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HAROLD H. STRAIN

CONCERNING THE pH MEASUREMENT OF COLLOIDAL SYSTEMS
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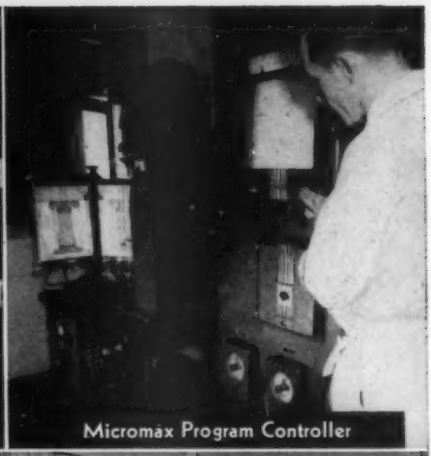

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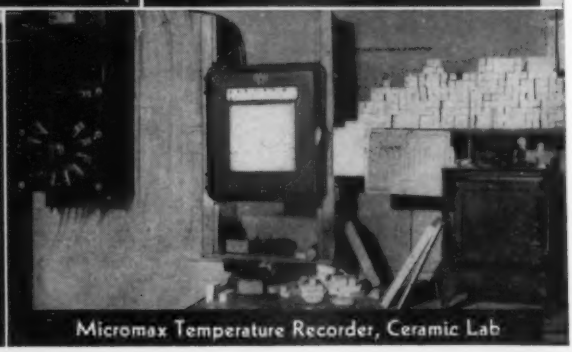

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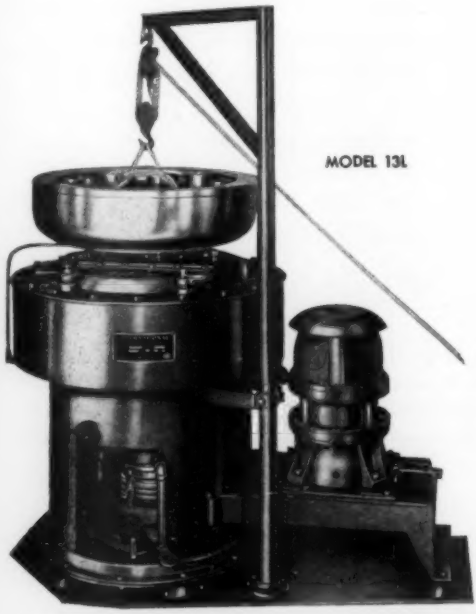

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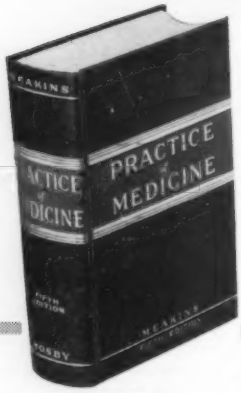

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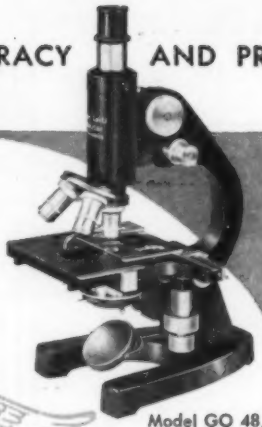

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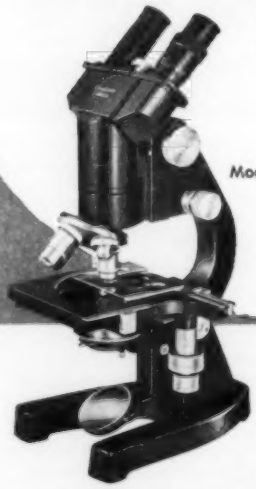

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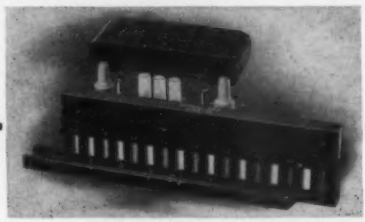

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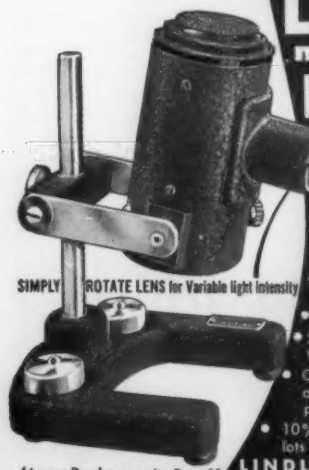

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Cellular Opacity and the Activity of Chloroplast Pigments in Photosynthesis

Harold H. Strain

Division of Plant Biology, Carnegie Institution of Washington, Stanford, California

IN GREEN PLANTS, chloroplast pigments utilize the energy of sunlight for the production of oxygen and organic matter. The effectiveness of this unique photosynthetic reaction varies greatly in light of different wavelengths. This variation of photosynthetic activity with wavelength was observed first when plants plus motile, oxygen-sensitive microorganisms were placed in a visible spectrum (Engelmann) (*7*). It has been demonstrated repeatedly by numerous comparative measurements of photosynthesis in different spectral regions (*1, 4, 10–13, 16, 25*) and by careful physical measurements of photosynthetic efficiency (*3, 5, 8, 22–24*). Thus far, variation of photosynthetic efficiency with wavelength has been attributed primarily to variation in the amount of light absorbed by active pigments and to differences among the intrinsic reactivities of the individual pigments.

The diversity of the chloroplast pigments has presented serious obstacles to precise determinations of their photosynthetic effectiveness. Some five dozen or more of these pigments have been isolated from various autotrophic organisms. Several to a dozen or more occur in each plant. Fortunately, most of the chloroplast pigments are of two principal kinds, the chlorophylls and the carotenoids. In certain algae, red and blue proteinaceous phycobilins accompany the carotenoids and chlorophylls. Only one pigment, chlorophyll *a*, is a major component of the chloroplasts of virtually all autotrophic plants (*17–19*).

As shown by many investigations, photosynthetic efficiency is greatest in the spectral regions that are strongly absorbed by the active chloroplast pigments, and it is least in the spectral regions that are weakly absorbed by active pigments. The dependence of photosynthetic efficiency upon the absorption capacity of the pigments has been encountered when a single active pigment as a chlorophyll (*6*), a carotenoid (*3*), or a phycobilin (*5, 8, 9*) is the principal light-absorbing agent. This effect has also been observed when chlorophylls plus phycobilins (*4, 7, 9–14, 21*) or chlorophylls plus carotenoids (*3, 4, 6, 11–13, 22, 23*) are the active photosynthetic pigments, and it has been found in dense cell suspensions that absorb all the incident light (*2, 3, 5, 6, 9, 11, 22, 23*).

Dependence of the photosynthetic efficiency upon the absorption capacity of the pigments may now be correlated, in part, with the amount of light absorbed by active pigments relative to that absorbed by nonactive, opaque substances. Absorption by pigments is, in turn, a function of the concentration and the condition of the pigments in the chloroplasts. It is also a function of the volume of the chloroplasts relative to the volume and the opacity of the plant cells.

With high absorption capacity by active pigments, the incident light traverses little of the plant material before it is absorbed. Under these conditions only a small proportion of the incident energy is lost to inactive cellular constituents, such as cell walls, food-storage granules, nuclei, protoplasmic colloids, refractive interfaces, inorganic ions, and inactive cytoplasmic pigments. With low absorption capacity by active chloroplast pigments, the light traverses much cellular material before it is absorbed; hence a much larger proportion of the incident energy is lost to nonreactive, opaque materials, and the photosynthetic efficiency is reduced proportionately.

Absorption Capacity of Individual Pigments and Photosynthetic Efficiency

Examples of the dependence of photosynthetic efficiency upon the absorption capacity of the active pigments have frequently been reported. In the green alga *Chlorella*, utilization of incident light by chlorophylls *a* and *b* is greatest in the spectral region of their absorption maxima but decreases at shorter and at longer wavelengths (*5*). In the diatom *Nitzschia* and in many brown algae, utilization of the light that is strongly absorbed by the carotenoid fucoxanthin is more efficient than utilization of light that is weakly

absorbed by this carotenoid pigment (*3, 10–13, 16, 23, 24*). In many red algae, utilization of the yellow-green light that is most absorbed by phycoerythrin plus chlorophyll is more effective than utilization of the red light that is less absorbed by the chlorophyll alone (*1, 3, 7–11*). In the blue-green alga *Chroococcus*, photosynthetic efficiency is likewise greatest in the spectral regions that are strongly absorbed by chlorophyll *a* plus phycocyanin, and it is least in the regions that are weakly absorbed by chlorophyll alone or by phycocyanin plus carotenoids (*5*).

In the blue-green spectral regions, absorption by carotenoid pigments is usually greater than the low absorption by chlorophylls (*5, 6, 9, 11, 17, 22*); hence low photosynthetic efficiency in this region of weak absorption has commonly been attributed to low specific activity of the carotenoids (*5, 6, 22*). As this low photosynthetic activity may now be ascribed, in part, to a greater loss of the weakly absorbed light to nonreactive substances in the plant cells, the photosynthetic effectiveness of the carotenoids must be greater than was thought. Examination of this possibility depends upon knowledge of the concentration and the spectral absorption properties of the pigments, as well as upon knowledge of the opacity of the cellular material.

Concentration of the Pigments and Their Absorption Capacity

The relative absorption capacity of the pigments at different wavelengths varies not only with their spectral absorption properties but also with their concentration (*13*). Chlorophyll *a*, the principal chloroplast pigment of most autotrophic plants, exhibits great variation in its absorption capacity for green light in comparison with red light (*19*). The higher the concentration of the chlorophyll, the greater the absorption capacity for green light relative to the absorption of red light. For a methanol solution with a light path of 5 microns and with a chlorophyll concentration of 2 percent, just over 80 percent of the incident blue and red light will be absorbed, but only 4 percent of the incident green light will be retained. Virtually all the incident red and blue light could, therefore, be absorbed by a single chloroplast containing this amount of chlorophyll, whereas most of the incident green light would be transmitted to penetrate other cellular materials (*18, 19*). Even with a chlorophyll concentration of 10 percent and with a light path of 10 microns, the absorption of green light by a single chloroplast would still be less than one-third that of red light. In the largest and greenest chloroplasts, without this much chlorophyll, the incident green light would penetrate many chloroplasts and much cellular material before it is absorbed.

Condition of the Pigments and Photosynthetic Efficiency

Calculation shows that absorption of light by pigments relative to the absorption by nonphotosynthetic substances depends upon the volume of the chloroplasts relative to the volume and the opacity of the cells. For a given amount of chlorophyll, the smaller the volume of the chloroplasts relative to the cells, the lower the absorption by the active pigments and the greater the proportion of incident energy lost through absorption by nonreactive materials. In harmony with this conclusion, the highest photosynthetic efficiencies have been found in unicellular plants rich in pigments and with large chloroplasts filling most of the cells (*3, 5, 6, 20, 22*). The lowest photosynthetic efficiencies have been found in plants with a few small chloroplasts relative to the total amount of plant material (*2, 11*).

Absorption of light by active pigments relative to absorption by inactive substances also depends upon the condition or distribution of the pigments within the chloroplasts (*19*). The more diffuse the distribution, the greater the absorption by the pigment relative to the absorption by other less active pigments and by inactive substances. As indicated by the effect of heat and protein reagents upon the color of algae, the water-soluble proteinaceous phycoerythrin is diffusely distributed through the chloroplasts of red algae, and the similar phycocyanin is similarly distributed in the pigmented portions of blue-green algae (*19*). Qualitatively, there is a positive correlation between the condition of these phycobilin pigments in the cells and the photosynthetic efficiency (*8, 9, 11*).

Cellular Opacity and Photosynthetic Efficiency

Absorption spectra of leaves, of bleached chlorophyllous tissue (*15, 17, 23*), and of pigment-free cells show that the absorption of light by the nonphotosynthetic components of plants varies rather uniformly with wavelength. Absorption is greatest in blue light and least in red. Qualitatively, therefore, the utilization of light by active pigments depends primarily upon their absorption capacity. Quantitatively, the utilization of light by active pigments depends upon their intrinsic photosynthetic activity and upon their absorption capacity relative to the total absorption capacity of the plant material.

Many qualitative observations and a few quantitative measurements indicate that great opacity of the plant cells is associated with low photosynthetic efficiency. Leaves that contain large amounts of opaque structural material utilize the incident light very inefficiently (*11, 15, 17*). In shallow-water brown algae of the family Fucaceae, great opacity is associated with low photosynthetic activity. In deep-water

brown algae, by contrast, low opacity permits efficient utilization of the incident light (*11–14, 17*). As a rule, the highest photosynthetic efficiencies have been found in unicellular algae that exhibit great absorption capacity by active pigments and low absorption capacity by their cellular substance (*2, 5, 6, 11, 23*).

In leaves the quantitative relationship between absorption by nonreactive substances and absorption by active chloroplast pigments varies enormously with the leaf itself and with the wavelength of the incident light. In the green spectral regions, where absorption by active pigments is at a minimum, as much as 20–50 percent or more of the light may be lost to nonreactive, cellular substances (*11, 15, 17*) that occur outside the chloroplasts (*15*). But at wavelengths that are strongly absorbed by the chlorophylls, only 1–5 percent of the incident light may be lost (*15, 17*).

From these considerations, cellular opacity has a large effect upon the photosynthetic efficiency only in spectral regions of low absorption capacity by active pigments. Even in these regions photosynthetic efficiency is related to the concentration of the active pigments, to the condition of the pigments in the chloroplasts, and to the number, size, and orientation of the chloroplasts. Owing to the natural variation of all these conditions and to the complexity of the pigment mixtures, evaluation of the photosynthetic effectiveness of the individual pigments in different plants is subject to great uncertainty.

Relative Effectiveness of Individual Chloroplast Pigments

As indicated already, the photosynthetic effectiveness of the individual pigments in each organism has been estimated from relative or comparative measurements of photosynthesis at different wavelengths (*11, 22*). For these comparisons the intrinsic activity of each pigment has been assumed to remain constant, irrespective of the absorption capacity. When corrected for the light lost to opaque substances, the differences among the activities of the several pigments are reduced. An example cited above is the greater effectiveness of the weakly absorptive carotenoids in higher plants, in green algae, and in blue-green algae. Similarly, the effectiveness of the abundant phycoerythrin in red algae (*8, 9, 11*) is reduced relative to the effectiveness of the chlorophyll.

In *Chlorella* photosynthetic efficiency of chlorophylls *a* and *b* in weakly absorbed red light (700 mμ) is very low relative to the efficiency in strongly absorbed red light (680 mμ) (*6*). This effect may now be ascribed to a proportionately greater loss of the weakly absorbed light to nonreactive materials, rather than to variation of the photosynthetic efficiency of the green pigments (*6*).

In diatoms and in deep-water brown algae, the photosynthetic efficiency in the spectral range of 500–600 mμ approaches that in red light (*3, 10–14, 16, 23, 24*). As chlorophyll *c* absorbs much of the incident light in this spectral region (*3, 20*), the pigment must be effective in the photosynthetic process. This conclusion and the activity of diatom carotenoids support the view that deep-water plants deriving their energy primarily from light of low intensity cannot afford high concentrations of noneffective pigments that absorb strongly in this spectral region (*19*).

Pigments and the Mechanism of Photosynthesis

The photosynthetic effectiveness of the chloroplast pigments is more intimately related to their spectral absorption capacity than to any other established physical or chemical property. Aside from their great spectral absorption capacity, the diverse pigments of the chloroplasts do not have many chemical properties in common. Thus far, the use of the most sensitive chromatographic adsorption methods has not revealed colored alteration products of the chloroplast pigments in photosynthesizing plants (*18*). Yet, if one molecule of chlorophyll were involved in the transfer of two hydrogen atoms or of one oxygen atom per molecule of assimilated carbon dioxide, some 30 grams of chlorophyll would undergo change per gram of carbohydrate produced. In view of these facts, the transformation of radiant energy to chemical energy during photosynthesis must depend upon instantaneously reversible reactions of the pigments, or upon reactions confined to colorless components of the pigment-chloroplast system.

In the utilization of sunlight by chloroplast pigments, chlorophyll *a* plays a predominant role. It is the most abundant and, in many plants such as the yellow-green algae, it is the only green chloroplast pigment. Chlorophyll *a* has great spectral absorption capacity; it is effective in spectral regions where carotenoids do not absorb light; and it exhibits great photosynthetic efficiency. As a consequence, elucidation of the mechanism of energy transfer in autotrophic plants hinges upon knowledge of the activity of this chlorophyll.

The efficiency of photosynthesis in monochromatic light varies with the spectral absorption capacity of the pigments and with the opacity of the plant cells. With high pigment concentration, with great spectral absorption capacity, with large chloroplasts, and with transparent plant cells, little of the incident energy is lost to nonreactive opaque substances, and the efficiency of photosynthesis is high. With low spectral absorption capacity, with a small volume of chloroplast material relative to the volume of the cells, and

with opaque plant material, a large proportion of the incident energy is lost to the inactive opaque substances, and the photosynthetic efficiency is low.

When the opacity of the plant material is considered as a factor affecting the efficiency of photosynthesis, most chloroplast pigments must be regarded as effective, though not necessarily equally effective, in the process. The available evidence suggests that energy absorbed by pigments is utilized without concomitant chemical change of the pigments themselves.

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Concerning the Measurement of pH, Ion Activities, and Membrane Potentials in Colloidal Systems

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IN THE FIELDS of biology and agriculture and in many industrial laboratories, pH measurement of colloidal systems, such as gels and thick suspensions, plays a prominent role in research and testing activities.

Measurement of pH with H_2-, quinhydrone-, and glass-electrodes, and of cation activity with various charged membranes, always involves a liquid junction, usually at a KCl bridge. In measurements made in true solutions the liquid junction potential probably is negligible, but this is not necessarily true for systems containing a great number of electrically charged particles of extremely high valencies, such as those represented by large clay and resin particles.

We are proposing a theory, supported by experimental data, showing that in certain colloidal systems liquid junction potentials may assume considerable magnitudes.

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Consider microscopic particles of cation exchange material (e.g., resin) bathed in a dilute solution of KCl. Let ionic equilibrium be established. When a salt bridge containing saturated KCl is inserted into such a system, a junction between solutions having two different KCl concentrations within the matrix of the negatively charged particles results. If the negatively charged particles affect the relative diffusion rates of K^+ and Cl^-, a potential will arise at the interface of bridge and assemblage of particles, which we shall describe in terms of the activities of KCl in the liquid between the particles and in the bridge and the transference numbers of K^+ and Cl^- in the interface.

Transferenee numbers of K^+ and Cl^- in systems comprising KCl solution and cation exchanger material were determined by a modification of the Hittorf method. An 8-mm layer of the cation exchange material was interposed between the anode and cathode compartments of a Bradfield cell so that ions trans-

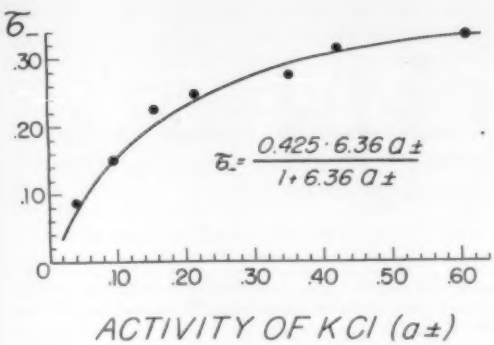

FIG. 1. Transference numbers of Cl in the system K-Ion-X + KCl.

porting current had to pass through the exchange material. The two membranes were prepared from uncharged Visking cellulose casings. Transference numbers ($\tau-$) so determined in systems containing a variety of cation exchange materials are presented in Fig. 1 and in Table 1. It is apparent that the negatively charged particles seriously affect the relative movements of Cl^- and K^+. The higher the charge density of the solid phase, as reflected by cation exchange capacity, the smaller is τCl^-. As seen in Fig. 1, the influence of the ion exchanger on $\tau-$ is greater at low than at high electrolyte concentrations.

Potentials developed across a layer of cation exchanger material in equilibrium with KCl solution of a given concentration C_1, and separating this first solution from a second KCl solution of a different concentration C_2, were measured (Table 1). The cell was:

$$\mathrm{Hg}\left|\mathrm{Hg_2Cl_2}\right|\mathrm{KCl\ (sat)}\left|\mathrm{KCl}\ \underset{(C_2)}{(a=a_2)}\right|\underset{X}{\text{cation exchanger}}\left|\underset{Y}{\text{+KCl}\ (C_1)}\right|$$

$$\mathrm{KCl}\ \underset{(C_1)}{(a=a_1)}\left|\mathrm{KCl\ (sat)}\right|\mathrm{Hg_2Cl_2}\left|\mathrm{Hg}\right.\quad \text{Cell A}$$

Either calomel electrode can be replaced by a glass electrode without affecting changes in emf observed when a_2 is changed. As will be discussed later, the equality of the Cl concentration (C_1) on both sides of the membrane Y was verified experimentally.

The general form of the equation for the liquid junction potential between two solutions I and II, is:

$$E_L = \frac{RT}{F}\int_{\mathrm{I}}^{\mathrm{II}} \Sigma_i \frac{\tau_i}{z_i}\, d\ln a_i \qquad (1)$$

where τ_i, z_i, a_i represent transference number, valency, and activity of a given ion species. For the case of KCl, using the τ-a-function depicted in Fig. 1 and assuming that the activity, if KCl is not affected by the cation exchanger, $a_+ = a_- = a_\pm$, the integrated equation assumes the form:

$$E_L = \frac{RT}{F}\left[\ln\frac{a_2}{a_1} - 0.85\ln\frac{1+6.36\,a_2}{1+6.36\,a_1}\right] \qquad (2)$$

where a_1 and a_2 refer to mean activities of KCl. When a_1 and a_2 do not differ greatly, so that τ_i may be considered to a first approximation to be independent of concentration, equation (1) becomes:

$$E_L = (\tau_+ - \tau_-)\frac{RT}{F}\ln\frac{a_2}{a_1} \qquad (3)$$

According to Table 1, the agreement between measured emf and calculated emf (equation 2) is satisfactory, even for fairly large concentration differences. Although the boundary conditions at the KCl junction (X) are undoubtedly more complicated than is assumed in equation (1), the order of magnitudes of the high potentials observed (Table 1) are predictable on the basis of a liquid junction potential.

TABLE 1

JUNCTION POTENTIALS IN COLLOIDAL SYSTEMS

Exchanger	Cation exchange capacity me per 100 gm	Mean activity of KCl solution		Junction potential millivolts		
					Calculated	
		a_1	a_2	Measured*	eq.2	eq.3†
Ion—X	370	0.00463	0.00902	18.0	17.7	17.0
Dow Chemical Corp.		"	0.0441	53.7	53.7	57.8
		"	0.296	91.7	85.2	106.8
		"	0.432	96.5	89.3	116.3
		"	2.323	113.0	102.0	159.5
IR—100	173	0.0408	0.0746	11.0	....	10.0
Resinous Products Co.		"	0.462	38.2	....	40.0
Bentonite	100	0.0408	0.0751	5.9	....	5.0
Utah		"	0.454	20.4	....	21.0

* Measured with Cell A.

† Using the experimentally determined value of τ_1 referring to a_1. For $a_{KCl} = 0.0408$; $\tau-$ for IR − 100 = 0.180; $\tau-$ for bentonite = 0.327.

The interpretation presented is at variance with the customary explanation of the emf measured with Cell A. It is often assumed that the seat of the emf is not at the junction X but at the boundary Y, which is said to act as a membrane of the Donnan type. Thus the emf observed is attributed to a Donnan membrane potential at Y, rather than a junction potential at X. The latter is considered to be negligible, especially if the KCl solution (C_2) is saturated. At present it is impossible to distinguish by direct emf observation between the two theories, because any emf measurement includes both potentials. Both potentials have the same sign.

Our investigation on transference numbers indicates that the potential difference at the junction X may be appreciable, at least for the systems under consideration (amberlite, Dowex, clays, soils). Moreover, our theory predicts the correct order of magnitude for the emf, provided the potential difference at Y is considered to be very small. It is important to note that, if one wishes to adhere to the theory of membrane potential at Y, it is necessary to conclude that its magnitude depends on the KCl concentration in the salt bridge as indicated by C_2 in Cell A, and a_2 in equation (2).

In ordinary potentiametric pH determinations, the usual cell is:

$$\mathrm{Ag}\,|\,\mathrm{AgCl,\ HCl}\,|\,\mathrm{Glass}\,|\,\begin{matrix}\text{Test}\\ \text{solution}\\ \text{KCl},\ a=a_1\\ \text{H},\ a=x\end{matrix}\,|\,\underset{X}{\underset{a_2}{\mathrm{KCl\ (sat)}}}\,|\,\mathrm{Hg_2Cl_2}\,|\,\mathrm{Hg}$$

It is generally assumed that the liquid junction potential, test solution KCl $a_1|$KCl (sat), at X, is negligible, and that only the emf across the glass membrane is measured. If, however, the solution contains many suspended or sedimented electrically charged particles of high valency, this system corresponds to that depicted in Cell A. The emf measured includes both the potential across the glass and the potential arising at the junction between the KCl bridge and the system containing particles. The latter emf may be estimated on the basis of unequal transference of K^+ and Cl^- ions in the test system, in contrast to equal transference of these two ions in true solution, and to differences in KCl concentration between the salt bridge and the intermicellar liquid.

Generally speaking, the junction potential operates in the direction of making the pH of suspensions, or particularly of pastes, appear lower than is attributable to the H ion activity of the system. The following striking examples are presented: a K-Ion-X containing no exchangeable H ions (with $\frac{n}{1}$ KCl leaching) will, when immersed in water, give a pH of 2.8 when the electrode pair of a Beckman assembly is pushed into the coarse sediment. A K-bentonite having a pH of 9.2 in a 10 percent suspension will give a pH of 5.8–5.9 if the electrode pair is inserted into a paste consisting of equal volumes of bentonite and water.

The simple case that has been developed in equation (1) and Table 1 involves an ion exchanger KCl solution system at pH 7, so that KCl is the only electrolyte to be considered in the calculation of the emf expression. For more complex systems, additional terms must be inserted in the emf equation to account for the contribution of other ion species to the junction potential.

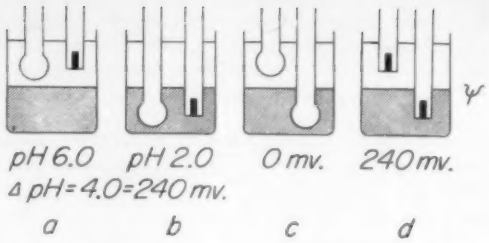

FIG. 2. Illustration of suspension effect (Δ pH) and "membrane potential." Glass electrode symbolized by sphere, calomel electrode by rectangle with a black bar representing the KCl-bridge.

The theory that has been briefly developed here sheds interesting light on some important observations relating to the *suspension effect*, viz., the difference in pH of a colloidal system (suspension, gel) and its filtrate, supernatant liquid or intermicellar liquid. It has long been observed that, in a sediment of acid soil in equilibrium with its supernatant liquid, the pH of the sediment is considerably lower than that of the supernatant liquid.

Du Rietz (*3*) and Davis (*2*), among others, have found that the pH of such a two-phase system depends not at all on the position of the glass electrode in the system. However, the position of the salt bridge leading to the calomel electrode, whether in the sediment or in the supernatant liquid, has a profound effect on the pH values obtained. An extreme example of this phenomenon may be obtained with the H-resin Ion-X dispersed in water. A supernatant liquid with a pH of 6.0 in equilibrium with a sediment of pH 0.5 may be found. The difference, Δ pH, corresponds to the very large emf of 325 millivolts.

Experiments in this laboratory have shown that variations in the KCl concentration of the salt bridge or variations in the electrolyte concentration of the suspension result in systematic changes in the pH difference between sediment and supernatant liquid. The emf values obtained may be related to electrolyte concentrations in the bridge and in the suspension by equations similar to equation (1). In all such systems, the magnitude of the suspension effect (Δ pH) corresponds exactly to the potential across two calomel electrodes with KCl salt bridges, one immersed in each phase (Fig. 2 *d*).

If, on the other hand, electrodes reversible to hydrogen ions are inserted in the supernatant liquid and in the sediment, and connected to a voltmeter, no emf results (Fig. 2 *c*). Its absence is usually explained by a Donnan membrane potential "without membrane"

existing at the boundary of sediment and supernatant liquid. The magnitude of the "membrane" potential is said to be equal and opposite to the suspension effect. According to Brönsted (*1*), "the membrane potential is measurable as the difference between the electromotive potentials of two standard electrodes (e.g., calomel electrodes) in contact with the solutions on the two sides of the membrane, the junction potential being eliminated." Following Loeb (*4*), the membrane potential is often calculated from the difference of the pH values observed potentiometrically between "inside" (sediment) and "outside" solution.

With numerous resin and clay systems we have verified the identity of the "membrane" potential and the Δ pH values (suspension effect). However, our explanation is different. According to our picture we have merely measured the same junction potential twice, once when measuring pH and once when measuring the "membrane" potential (Fig. 2, *a*, *b*, *d*).

It is important to note that in our systems

$$\mathrm{KCl}\,|\,\text{K-cation exchanger}$$

$$\mathrm{C_1}\,\Big|_{Y}\,\mathrm{KCl}$$

no Donnan distribution across the interface, *Y*, could be observed. Even after long standing, the Cl^- concentration of solution removed by rapid filtration from the right-hand compartment was identical with that of the solution in the left-hand compartment. Likewise, if colloidal H-resin particles are added to one side of a two-compartment cell containing equal amounts of HCl on both sides, no redistribution of HCl takes place. This suggests that *Y* is not a phase boundary in the usual sense, and that the solution phase KCl (C_1) exists on both sides of *Y*. No membrane potential in the Donnan sense is expected to exist. Yet, by inserting two calomel electrodes with salt bridges, large emf values are obtained. These we attribute to the junction potential.

The following question still remains: If the boundary of supernatant liquid|sediment is not the chief seat of the potential difference observed between two calomel electrodes (Fig. 2 *d*), how is the absence of an emf between two glass electrodes (Fig. 2 *c*) to be explained? Two explanations come to mind. First, the activities of the H ions are identical in the supernatant liquid and in the sediment; this would imply that the H ions of the ion swarm of the particles have the same activity as those of the intermicellar liquid. Second, the adsorbed H ions (swarm) do not affect the glass electrode. Only the diffusable H ions of the intermicellar liquid are recorded by the electrode.

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Technical Papers

Chemical Composition of the Vitelline Membrane of *Ascaris lumbricoides* var. *suis*[1]

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A large number of living female *Ascaris lumbricoides* var. *suis* were slit open and the uteri dissected out. The edge of a glass slide was rubbed gently along the uteri to force the eggs out on a glass plate. The eggs at the distal end of the uteri were frequently checked under the microscope to make certain that they were fertilized. Immediately after collection the eggs were placed in a 0.2% solution of concentrated CP hydrochloric acid to dissolve the outer mucoid coat. The material was allowed to stand overnight at room temperature and was then centrifuged, and the supernatant fluid was stored in the refrigerator for further testing. The eggs were washed three times in distilled water and then exposed to artificial gastric juice at 37° C. This step was to eliminate any remnants of the mucoid layer. The supernatant fluid after this procedure showed no quantitative or qualitative differences from artificial gastric juice. This indicates that the previous hydrochloric acid extraction was complete.

The eggs were then rinsed and placed in a large test tube in a water bath and the temperature was held between 72° and 74° C in a warming oven. The vitelline membrane melted at this temperature and gathered in large globules, usually at one end of the egg. The eggs were allowed to cool and an acetone extraction for fats and fatty acids was made at room temperature. The supernatant fluid was filtered off, and the extraction was repeated three times. On chilling, this supernatant fluid showed a white flocculent precipitate. Later tests with heated beeswax in acetone showed a similar precipitate on chilling. The nature of the precipitate in our acetonic

[1] This work was carried out under the guidance of B. G. Chitwood.

extract of vitelline membrane has not as yet been investigated, but it seems probable that some of the vitelline substance was dissolved at the temperature of the extraction.

The eggs were then exposed to ethylene dibromide, the supernatant fluid from this procedure being thereafter drawn off and placed in a dish and evaporated to dryness. A waxlike substance remained on the bottom of the crucible. Neither the acetonic nor the ethylene dibromide extractions gave positive ninhydrin or biuret reactions for protein, whereas the eggs after the ethylene dibromide extraction gave strong positive reactions.

The waxlike substance produced by the ethylene dibromide extraction is the material which formed the vitelline membrane in the original living egg. This substance was tested for the presence of an ester (R_1COOR_2) in the following manner. A drop of the ethyl ethereal solution of the vitelline membrane was placed in a microcrucible. To this were added a drop of an alcoholic solution of hydroxylamine hydrochloride and a drop of alcoholic sodium hydroxide. The crucible was heated over a flame until there was a slight effervescence. The solution was acidified with 0.5*N* hydrochloric acid and a drop of 1% ferric chloride was added. Reddish-yellow lumps formed. Upon evaporation pyramidal crystals (with a square base) were formed. Testing under a microscope with polarized light showed that these crystals were nonbirefringent, but on their surface a few minute, elongate, birefringent crystals were observed. We are not entirely satisfied with this test.

A control of the above test was run with a known ester, myricyl palmitate (beeswax), and an identical reaction was obtained. Crystal formation was not observed, but the presence of impurities was shown by the low melting point (65° C). U.S.P. beeswax (chiefly myricyl palmitate) was obtained for comparison and further refined by dissolving in warm acetone, chilling, and centrifuging. The melting point of the product was found to be 66.2° C. The melting point of pure myricyl palmitate is given as 72° C. The vitelline membrane wax produced by ethylene dibromide extraction was tested for melting point; it began to melt at 69° C and ran slowly at 72° C. In the living egg the vitelline membrane does not begin to melt until the temperature 70° C is reached and it melts completely at or below 73° C. Hence our product is not as pure as that produced by the nematode. Equal amounts of the vitelline membrane wax and the refined beeswax were mixed together, the mixture was fused at 75° C and cooled to 58° C, and thereafter the temperature was raised 1° C at 5-min intervals. This mixture began to melt at 68° C and ran slowly at 70° C. Such evidence is usually accepted as conclusive proof of the identity of the two principal ingredients of the mixture. If the principal ingredients had not been identical, the mixture should have had a melting point below either of the original ingredients. Instead, the melting point was higher than one ingredient and lower than the other. One would judge from these facts that the refined commercial U.S.P. beeswax contained impurities not present in the nemic vitelline membrane wax. Upon the basis of all of this evidence, we feel justified in concluding that the vitelline membrane in the living egg of *Ascaris lumbricoides* var. *suis* is myricyl palmitate.

The myricyl palmitate as produced by our ethylene dibromide extraction was softer than commercial beeswax and took longer to harden. There may be some stratification on cooling. The latter point will require further investigation. The myricyl palmitate produced by precipitating the hot acetonic extract of either the commercial grade or the U.S.P. grade of beeswax is of a higher degree of purity than the original material. It seems possible that one might be able to produce a completely pure compound by repeating this process an adequate number of times.

This is the first paper presenting proof of the nature of the vitelline membrane in the Nematoda. Since the melting point, solubility, and other characteristics of the vitelline membrane as previously determined in *Meloidogyne hapla*, *M. javanica*, *Parascaris equorum*, *Rhabditis strongyloides*, *Ditylenchus dipsaci*, and *Strongyloides canis* do not differ materially, it seems probable that the vitelline membrane in these organisms is the same or a similar compound. This may be the first paper presenting evidence of a wax in unsegmented animals.

Effect of Trace Minerals on Growth and Fattening of Swine [1]

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The beneficial effects of cobalt for ruminants in areas of cobalt deficiency have been demonstrated. Knowledge is limited as to the value of trace minerals, particularly cobalt, for swine. Willman and Noland (*5*) reported increased weight gains for swine receiving four minerals—cobalt, copper, iron, and manganese—when added to a corn-soybean oil meal ration in dry lot.

Rickes *et al.* (*4*) showed that vitamin B_{12} contains cobalt. Recently, Abelson and Darby (*1*) demonstrated that cobalt is used in the synthesis of vitamin B_{12} by rumen bacteria. Becker, Smith, and Loosli (*2*) reported, however, that on the basis of preliminary observations, administration of vitamin B_{12} did not altogether relieve symptoms of cobalt deficiency in sheep, and that cobalt was probably required for other body processes in the ruminant.

For the initial study of the effects of cobaltized and trace-mineralized salt on pigs, a ration was formulated using ground yellow corn, ground barley, linseed oil meal, tankage, ground alfalfa, steamed bone meal, and salt. These pigs were kept in concrete pens, which were washed daily. Treatments and results are presented in Table 1.

Two more experiments were conducted to study the effect of cobalt on the growth and fattening of swine

[1] North Dakota Agricultural Experiment Station Progress Report Project R&M 10, published with approval of director.

TABLE 1

EFFECT OF TRACE MINERALS ON WEIGHT GAIN AND FEED EFFICIENCY OF PIGS

Lot No.	I	II	III
Treatment	Iodized salt	Cobaltized salt*	Trace-mineral salt†
No. of pigs per lot	5	5	5
Initial wt, avg lb	52.6	53.2	51.4
Final wt, avg lb	163.8	184.2	192.6
Days on feed	98	98	98
Daily gain, avg lb	1.13	1.34‡	1.44‡
Feed in lb per 100-lb gain	431.3	389.0	394.9

* Cobalt chloride added to iodized salt to supply 0.026% cobalt.

† Trace-mineralized salt used contained the following amounts of minerals: manganese, 0.45%; iodine, 0.019%; cobalt, 0.026%; iron, 0.48%; copper, 0.048%; and salt, 96.0%.

‡ Differences from control lot significant at 5% level.

and its possible relationship to vitamin B_{12} (APF).[2] These pigs were self-fed on a basal ration of ground yellow corn, soybean oil meal, ground alfalfa, steamed bone meal, and salt. Lot IV received 8% meat scraps at the expense of soybean oil meal, and the corn was increased. Each lot was weighed at the end of the experiment when the lot average was approximately 180 lb. The pigs in these lots were fed in concrete pens, which were washed out daily. Table 2 summarizes these two experiments. The differences in gain are significant at the 5% level.

These results demonstrate that additions of cobalt to these rations stimulated weight gains more than additions of 8% of meat scraps and almost as much as the APF supplement. The differences in gains between lots I, II, and IV are not large enough to be significant but these differences were of about the same magnitude in both experiments. The results on feed per 100-lb gain were more erratic, with lots II and IV requiring slightly less feed per lb of gain.

TABLE 2

EFFECT OF COBALT, APF, AND MEAT SCRAPS ADDED TO A CORN, SOYBEAN MEAL, ALFALFA MEAL RATION FOR PIGS*

Lot No.	I	II	III	IV
Ration	Basal + cobalt†	Basal + APF‡	Basal	Meat scraps 8%
No. of pigs per lot	10	10	10	10
Initial wt, avg lb	28.7	28.7	28.7	28.8
Final wt, avg lb	180.5	180.8	176.8	181.4
Avg days to reach 180 lb	108	106	119	111
Avg daily gain, lb	1.40§	1.43§	1.24	1.37§
Feed in lb per 100-lb gain	377	365	375	358

* Average of two experiments.

† Salt (0.75 lb) containing 0.026% cobalt added per 100 lb of ration.

‡ Merck and Company supplied APF Supplement No. 3 that furnished about 9.42 μg vitamin B_{12} per lb of ration.

§ Differences from basal lot significant at 5% level.

[1] APF is the animal protein factor.

It is not known how cobalt functions. It is possible that cobalt is used in intestinal synthesis of vitamin B_{12}. Briggs (*3*) suggested, on the basis of preliminary studies, that under certain conditions cobalt is at least partially effective in counteracting a vitamin B_{12} deficiency in the chick. This action of cobalt is perhaps due to intestinal synthesis. It is less likely that the beneficial results of cobalt feeding in the present experiments were due to coprophagy, because the pens were washed daily and the pigs were never observed to be consuming feces. Neither is it probable that there was some synthesis in the feed mixture, although the ration was mixed in a spiral mixer in amounts to last 5–6 days.

Further research is under way to obtain additional information and to ascertain whether a combination of cobalt and APF, or of cobalt, APF, and meat scraps would further increase gains above that of any ingredient alone.

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Evidence of a Homing Instinct in the Bermuda Spiny Lobster[1]

Edwin P. Creaser[2] and Dorothy Travis[3]

During the summer of 1949 the authors,[4] in connection with various other investigations at the Bermuda Biological Station on the spiny lobster, tested local migration in this species by the use of tags or by telson and uropod markings. In the original studies, various combinations of uropod and telson punches were employed for identification, but subsequent use of plastic tags was undertaken for individual recognition. The plastic tags were made with a barb which penetrated into the muscle when thrust through the intersegmental membane between abnominal segments. The tag used was of the same general type developed by Smith (*1*) in the Florida and Caribbean spiny lobster investigation.

Intensive trapping operations were conducted through special arrangements with a Bermuda commercial fisherman, Egbert Spurling. The gear employed was a grappling hook and 20 fish traps, the latter of typical Bermuda design, made of chicken wire and supported by spicewood. The trapping operations were conducted from motorboats, and all work was conducted in the

[1] Contribution from Bermuda Biological Station No. 164.

[2] Hofstra College, Hempstead, New York, and Bermuda Biological Station, Bermuda.

[3] Radcliffe College of Harvard University, Cambridge, Massachusetts.

[4] The cooperation of all persons at the Bermuda Biological Station is acknowledged, especially that of Tommy Gleeson, Brunell Spurling, and Egbert Spurling.

TABLE 1

Date caught	Place released	Date recaptured	Remarks
7/21/49	Castle Harbor near causeway	7/25/49	13 released 3 recaptured
7/22/49	where caught	7/30/49	
7/21/49	Castle Harbor near causeway	7/30/49	
7/25/49	Castle Harbor near causeway	7/30/49	20 released 4 recaptured
7/21/49	Castle Harbor near causeway	8/2/49	
7/27/49	where caught	8/2/49	eggs shed when caught; when recaught, with newly laid eggs
7/22/49	where caught	8/2/49	
7/30/49	deep water, 2 miles off Castle Roads	8/4/49	released in water 1,500 ft deep
7/30/49	deep water, 2 miles off Castle Roads	8/6/49	released in water 1,500 ft deep
7/22/49	where caught	8/6/49	
7/27/49	where caught	8/11/49	
8/6/49	wharf of Frick estate on Castle Point, Castle Harbor	8/13/49	eggs when recaptured; newly shed when released
7/21/49	Castle Harbor near causeway	8/13/49	new eggs when recaptured; eggs when released
8/2/49	Castle Harbor near causeway	8/26/49	
7/21/49	Castle Harbor near causeway	8/26/49	
8/26/49	Ferry Reach at Biological Station jetty	9/28/49	

quarter-mile square outside area off Nonsuch Island, near the mouth of Castle Harbor. The waters in this area are rough and stormy, with swells, and have abundant coral heads and boiler reefs. Trapping operations were conducted throughout 2 months, and the traps were visited about twice a week.

Spiny lobsters, *Panulirus argus* (Latreille 1804), caught in this area were transported to various sites and released. The tagging experiments were considered as entirely preliminary in nature, but have produced such interesting results as to warrant discussion at this time.

In the first experiments on migration, the spiny lobsters were taken from the Nonsuch area and released 2 miles up Castle Harbor at a point in the middle of the harbor opposite Castle Harbor Hotel. Later experiments entailed releases behind land masses at various situations in Castle Harbor or in Ferry Reach, where the causeway largely obscures free passageway. Some lobsters were released where caught. In one experimental release the lobsters were carried 2 miles out to sea, where the waters were 1,500 ft deep, and were released there.

The dates and points of release and of recapture at the original site off Nonsuch Island are shown in Table 1.

The high incidence of recovery of specimens released at various sites (about 20%) indicates that we are probably concerned here with a remarkable homing instinct in these crustaceans. The recoveries of the specimens released in deep water 2 miles out at sea seem particularly significant. The return to the original site after release at the Biological Station jetty entailed migration against tides conflicting with those originally prevailing, and migration around land masses for about 5 miles.

It seems apparent that the lobsters are fully "aware" of their locations and can return to their original summer feeding grounds when released elsewhere. How this is accomplished remains an unanswered and puzzling biological mystery. The degree and nature of these local migrations are of very great scientific interest and equally important in a consideration of the management of the species commercially.

More detailed and extensive experimentation is planned for the future.

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Acetaldehyde Accumulation in Excised Wheat Roots Induced by Plant Growth Substances[1]

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It is commonly held that the observed effects of indole-3-acetic acid and other compounds of similar physiological activity must be referable to some basic influence of these compounds on the metabolism of the plant cell. Attempts to reveal this primary effect of the plant growth substances have led to postulated mechanisms concerned with the plasticity of the cell wall (*3*), a relationship between the 4 carbon dicarboxylic acids and auxin (*2*), a coenzyme function for auxin (*9*), a shunt mechanism for 2,4-D (*7*), and others (*1, 9*). Respiratory effects range from stimulation to inhibition (*6*), and may be insignificant at concentrations that have other marked effects (*7*). Current interest in compounds that inhibit pyruvate oxidation because of their similarity to a postulated 2 carbon derivative of pyruvate (*4*) suggested that a number of plant growth substances characterized by side chains of acetate or related to acetate might also affect pyruvate oxidation. Since a convenient and sensitive method for acetaldehyde exists (*11*) it appeared that the formation of this compound from added pyruvate would be a desirable subject to study.

Excised roots of 4-day-old wheat seedlings of the White Federation variety grown in redistilled water according to a technique previously described (*7*) were used. Each sample contained 24 roots cut to 45 mm in length. Before the roots were counted out, all the sections were

[1] This work was supported by a grant from the University of Illinois Research Board.

pooled and thoroughly mixed. It was found in some exploratory experiments that all but insignificant quantities of the acetaldehyde produced by the roots could be carried from the flask containing the roots to a receiving tube containing 2% sodium bisulfite by a continuous gas stream. Apparatus used in the experiments consisted of a battery of 25-ml Erlenmeyer flasks, each connected with rubber tubing to a receiving tube (18 × 150 mm). Acetaldehyde produced by the roots in each flask was carried over to the 2% sodium bisulfite solution in the receiving tube by an air or nitrogen stream. The air passed through a dilute NaOH solution and then through water before it entered the flasks. Nitrogen was used directly from the tank. The gas was passed through at a vigorous though undetermined rate. A second bisulfite receiving tube included on several occasions did not pick up acetaldehyde. This demonstrated that rate of gas movement was not too great to prevent complete absorption of the acetaldehyde. The flasks and receiving tubes were mounted on a shaking unit with the flasks in a water bath controlled at 25° C and the receivers in the air at room temperature. Shaking was at about 110 times a minute at an amplitude of 1 cm.

TABLE 1

ANAEROBIC PRODUCTION OF ACETALDEHYDE FROM PYRUVATE* AS AFFECTED BY CERTAIN GROWTH SUBSTANCES

Growth substance	Concentration moles/liter	Acetaldehyde/24 roots	
		µg	% control
Control	.....	8.5	100
Indole-3-acetic acid .	$1\ 10^{-4}$	11.25	131
Indole-3-acetic acid .	$1\ 10^{-3}$	18.25	209
Indole-3-butyric acid	$1\ 10^{-4}$	8.5	100
Indole-3-butyric acid	$1\ 10^{-3}$	14.0	164
Control	.....	5.85	100
2,4-Dichlorophenoxyacetic acid	$5\ 10^{-4}$	26.30	449
2,4-Dichlorophenoxyacetic acid	$1\ 10^{-3}$	6.70	114
Control	.....	5.75	100
Naphthaleneacetic acid	$1\ 10^{-5}$	5.65	98
Naphthaleneacetic acid	$1\ 10^{-4}$	7.50	130
Naphthaleneacetic acid	$1\ 10^{-3}$	17.15	298

* Pyruvate 0.0005M in all cases, adjusted to pH 6.0 with KOH except for naphthaleneacetic experiment at pH 4.8.

The earlier experiments of this study were done with the roots in an atmosphere of nitrogen and a solution of 0.0005M pyruvate, adjusted with KOH to pH 4.5 or 6.0, as indicated in the table. Results of several such experiments involving 4 compounds—indoleacetic, indolebutyric, naphthaleneacetic, and 2,4-dichlorophenoxyacetic acids—at various concentrations are shown in Table 1. All these compounds stimulated acetaldehyde accumulation.

It occurred to us that acetaldehyde production from added pyruvate might also be demonstrated in air if one were to add a sufficient amount of the acid. Very striking effects are produced by auxin, in fact, in 1 hr at pyruvate concentrations of 0.005 M (Table 2).

TABLE 2

THE EFFECT OF INDOLE-3-ACETIC ACID ON AEROBIC PRODUCTION OF ACETALDEHYDE FROM PYRUVATE*

Indole-3-acetic acid moles/liter	Acetaldehyde µg/24 roots
None	0
2.5×10^{-4}	0
5.0×10^{-4}	4.0
7.5×10^{-4}	7.5
1.0×10^{-3}	5.0
1.5×10^{-3}	5.25

* Pyruvate 0.005M in all cases, adjusted to pH 4.5 with KOH.

TABLE 3

AUXIN EFFECTS ON AEROBIC CONSUMPTION OF ACETALDEHYDE AND PRODUCTION OF ACETALDEHYDE FROM ENDOGENOUS MATERIALS

Indole-3-acetic acid moles/liter	Acetaldehyde, µg/24 roots		
	total	excess*	of endogenous origin†
None	0	....	0
2.5×10^{-4}	1.0	....	0
5.0×10^{-4}	20.75	7.0	0
1.0×10^{-3}	25.75	12.0	21.5
2.0×10^{-3}	19.0	6.25	6.0

* Acetaldehyde recovered in excess of the 13.75 µg added originally.

† A second experiment in which no exogenous substrate was added.

It is evident that the increased acetaldehyde production demonstrated in these experiments might be due (*1*) to an increase in the quantity of pyruvate metabolized or (*2*) to a diminished utilization of acetaldehyde. A solution containing 13.75 µg of acetaldehyde was added to root samples with varying amounts of indole-3-acetic acid. The inlet and outlet tubes of the flasks were clamped off, and the flasks were shaken for 1 hr. The flasks were then connected to the receiving tubes and air was passed through in the usual fashion for 1 hr. There is a marked inhibition of the utilization of the acetaldehyde (Table 3, center columns) by addition of auxin, but one also observes that at certain concentrations there is a greater quantity of acetaldehyde recovered than was originally added to the flask. It appears that the growth substance induced production of acetaldehyde from endogenous materials. Results of an experiment run under identical conditions except for the absence of exogenous substrate support this conclusion (Table 3, right-hand column).

Pyruvate may be metabolized through the tricarboxylic cycle or in a number of other possible ways which are referred to in Fig. 1 as "alternate pathways." It is recognized that the evidence for a tricarboxylic acid cycle in higher plants is not complete, but experiments of the sort reported by Machlis (*5*) appear to justify the introduction of such a cycle into the present discussion. At present one cannot definitely assign acetaldehyde a position either as an intermediate in the main pathway of aerobic carbohydrate metabolism or as a product of side

reaction. However, this seems to be of little consequence in the present discussion. Acetaldehyde accumulation (in either event) must be due to the inhibited utilization of pyruvate or one of the products in equilibrium with pyruvate (acetaldehyde, the postulated 2 carbon intermediate, or others). The inhibition would be either one that blocked the entry of pyruvate into the tricarboxylic acid cycle or it would be among all the other reactions that involve pyruvate. The former of these two general possibilities is the more appealing because several auxin effects appear to be due to diminished tricarboxylic acid cycle activity.

Work with respiratory poisons (*5*) suggests that energy released in the metabolism of the organic acids is used in salt accumulation. Inhibition of salt accumulation by indole-3-acetic acid, naphthalene acetic acid (*10*), and 2,4-D (*7*) may well be the result of diminished activity of the tricarboxylic acid cycle. Reversal of the 2,4-D inhibition of salt accumulation by citrate lends support to this idea.

Concentrations of growth substance that affect growth (*6*), and salt accumulation (*7*), do not appreciably influence respiration. This circumstance clearly indicates a qualitative change in the metabolism of the tissues. According to the present proposal, this lack of respiratory effect at low auxin concentrations is possible because

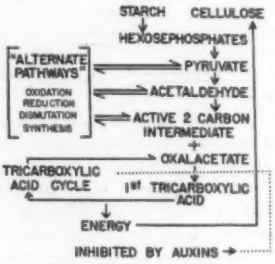

FIG. 1.

oxidations of the alternate pathways can consume all pyruvate (and its derivatives) blocked out of the tricarboxylic acid cycle. At higher levels of growth substance more pyruvate is available than the alternate pathways can utilize, so that there is an accumulation of acetaldehyde and an inhibition of oxygen consumption.

It is possible, by a somewhat less obvious argument, to relate certain growth effects of the auxins to diminished activity of the tricarboxylic acid cycle. This cycle is considered to represent perhaps the most efficient cellular mechanism for the release of chemical energy from carbohydrate. Energy is required for synthesis of the polysaccharides of the secondary cell wall from soluble precursors. Reduced activity of the acid cycle could then be associated with a restriction of these syntheses, with consequent maintenance of cell wall extensibility and capacity for further cell elongation. Increases in the soluble carbohydrates of tissues treated with indole-3-acetic acid (*8*) may also be explained in terms of an insufficiency of the chemical energy required for the synthesis of starch and other reserve carbohydrates from simple sugars.

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Antimycin A, an Antibiotic with Insecticidal and Miticidal Properties

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Antimycin A is a crystalline antibiotic isolated from cultures of an unidentified species of *Streptomyces*. It appears to be an optically active, nitrogenous phenol of the molecular formula $C_{28}H_{40}O_9N_2$ (*1*). Leben and Keitt (*2*) demonstrated the antibiotic properties of antimycin against certain phytopathogens and found that it is an extremely potent fungicide, producing inhibitory effects against *Nigrospora sphaerica* (Sacc.) Mason, for example, at dilutions as high as 1: 800,000,000. The present paper reports preliminary tests designed to investigate the insecticidal and miticidal potentialities of this material.

The initial trials showed that the antibiotic caused mortality to insects which ingested the material rather than by the contact action of the substance on the exoskeleton. For example, the common housefly *Musca domestica* L., sprayed with 10 ppm of antimycin A, showed no adverse effects, whereas 38% of the flies allowed to feed on a ball of absorbent cotton saturated with 10 ppm of antimycin A dispersed in water were killed in 24 hr. Similar results were obtained with the large milkweed bug *Oncopeltus fasciatus* (Dall.). Specificity was indicated, however, since certain insects, e.g., the German cockroach, *Blatella germanica* (L.), were able to ingest 10 ppm of antimycin A dispersed in water and live as long as those feeding on water alone.

The specificity of action was very apparent in a preliminary trial when the standard test wool fabric was immersed in a water dispersion containing 10 ppm antimycin A and offered to larvae of the webbing clothes moth, *Tineola bisselliella* (Hum.), and the black carpet beetle, *Attagenus piceus* (Oliv.). The larvae of the webbing clothes moth ate the test swatches with impunity, while duplicate test pieces inhibited the feeding of the black carpet beetle. Further tests were conducted with this beetle comparing antimycin A with sodium aluminum silicofluoride, which is widely used for fabric protection against insects. The standard wool fabric was

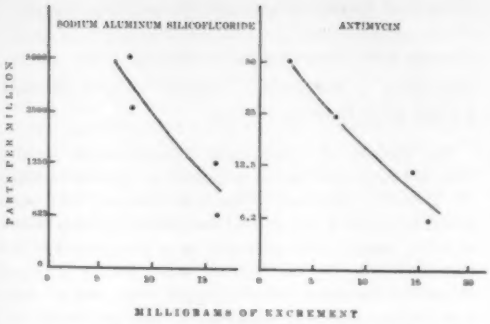

FIG. 1. Relative toxicity of antimycin A and sodium aluminum silicofluoride against larvae of the black carpet beetle. The amount of excrement produced by the larvae is related to the damage to fabric. A specimen is considered satisfactorily resistant to carpet beetles if the quantity of excrement is not over 6 mg.

immersed and saturated with the test subjects, aired, dried, and tested in accordance with the method set forth by the American Society for Testing Materials (ASTM D-582 45T). The data (Fig. 1) show that antimycin A will afford the same degree of fabric protection at 1/100 the concentration of sodium aluminum silicofluoride. The protective action appears to be of a repellent nature, as none of the larvae exposed to the antimycin-impregnated wool was dead at the end of the 28-day exposure period.

Specificity of action to Coleoptera and not to Lepidoptera was also indicated in tests with the Mexican bean beetle larvae, *Epilachna varivestis* Muls. and the Southern army worm, *Prodenia eridania* (Cram.). As in the case of the clothes moth larvae, the fourth instar Southern army worm ate treated Wood Prolific Lima-bean leaves with no ill effects. The results of the test with the second instar Mexican bean beetle larvae are shown in Table 1 and give the approximate relative potency of

TABLE 1

TOXICITY OF ANTIMYCIN TO SECOND INSTAR LARVAE OF MEXICAN BEAN BEETLE INGESTING SPRAYED WOOD PROLIFIC LIMA-BEAN LEAVES

Treatment	Ppm	Amount of leaf consumed	Avg % kill* in 48 hr
Antimycin	50	small	60
"	25	moderate	53
"	12.5	large	15
"	6.3	entire	0
Methoxychlor	500	moderate	43
Untreated	....	entire	0
Starvation	....	.....	0

* Avg of 4 replicates and total of 40 larvae.

antimycin A as compared with methoxychlor (1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane), which is currently used for the control of this pest. Antimycin A used at the rate of 25 ppm compares favorably in toxicity with 500 ppm of methoxychlor.

The toxicity of antimycin A is apparently not confined

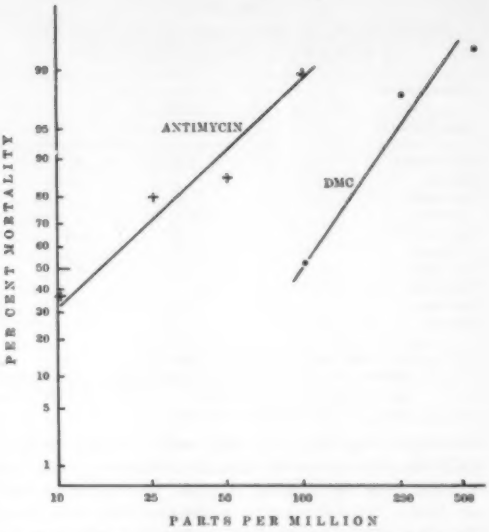

FIG. 2. Relative toxicity of antimycin A and di(*p*-chlorophenyl)methyl carbinol (DMC) against the red spider mite *Tetranychus* sp.

to members of the Insecta, as it shows efficacy for the control of the red spider mite, *Tetranychus* sp. Fig. 2 shows the relative toxicity of antimycin A as compared with di(*p*-chlorophenyl)methyl carbinol (DMC), which has demonstrated merit and is commercially available for mite control. The results of the test are shown graphically (Fig. 2). On the basis of LD_{50} readings, antimycin A appears to be about three or four times more effective than DMC.

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The Solubility of Fibrin Clots Produced by Thrombin and by Snake Venom

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It has been reported by Laki and Lóránd (*3, 4*) that there are marked differences among fibrin clots with respect to their solubility. The authors reported that fibrin clots formed from a fibrinogen solution by the coagulating action of thrombin and in the absence of two factors, namely, a thermolabile serum factor and Ca ions, are soluble in a concentrated urea solution. The clots are soluble when either of these two factors is absent but insoluble when both are present. The purpose of the present experiments was to find out whether clots formed by

TABLE 1

THE SOLUBILITY OF THE FIBRIN CLOTS PRODUCED BY THROMBIN OR BY *Bothrops atrox* VENOM

Components of the experiments	1*		2*		3*		4*		5*	
Fibrinogen	×	×	×	×	×	×	×	×	×	×
Serum	×	×								
Oxalated serum					×	×				
Inactivated serum									×	×
Ca ions	×	×					×	×		
Thrombin	×		×		×		×		×	
Venom		×		×		×		×		×
Result	−	++	+	+++	+	+++	+	+++	+	+++

* Symbols have the following meanings:
× marks in each column the components used in any one experiment; − insoluble; + soluble; ++ very soluble; +++ spontaneous solution.

the coagulating action of snake venom are influenced in their solubility by the two factors mentioned by Laki and Lóránd. The technique of the tests was similar to that of Lóránd, though smaller quantities were used than those mentioned by him (*4*). *Bothrops atrox* venom solution was used in such concentration as to give similar clotting times to those obtained by thrombin. The concentration of the urea solution was 60% and was added in volume equal to that of the reaction mixture; this mixture consisted of fibrinogen, the clotting agent, and one or two additional components, these being, in turn, serum, oxalated serum, serum inactivated by heat and $CaCl_2$ solution. When one of the additional components was excluded from the mixture saline was added to keep the volume of the mixture constant; a veronal buffer was used to maintain the pH of the mixture at 7.2. It was found that all the fibrin clots formed by the coagulating activity of the *Bothrops atrox* venom were soluble even though Ca ions and the serum factor were present. At the same time Lóránd's results were confirmed, since the clots formed by thrombin in the presence of the two factors were insoluble. The fibrin obtained by snake venom dissolved readily in all cases, and spontaneously when one of the two factors was absent. This cannot be stated of the clots formed by thrombin (Table 1).

Although, when observed with the naked eye, there seems to be no difference between the fibrin clots produced by either of the two agents, there probably is a difference in the degree of their polymerization, i.e., in their molecular structure. This might also explain the difference in their solubility.

The difference in the solubility of fibrin clots produced by thrombin and by venom offers further evidence in support of the opinion presented elsewhere (*1, 2*) that the clotting agent in the snake venoms of the *Bothrops* species is different from thrombin.

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Effect of Evisceration on Renal Glycogen

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The kidneys of eviscerated, hepatectomized rabbits, rats, and dogs were shown to contribute glucose to blood (*1, 2, 4–6*). Although it has been assumed that renal gluconeogenesis is the process responsible for this source of blood sugar, renal glycogen as a precursor has not been excluded. By determining serial kidney glycogen values in eviscerated, hepatectomized dogs, one of these possibilities could be excluded. If original renal glycogen values were great enough and sufficient renal glycogen disappeared, this storage form of carbohydrate could be assumed to play some role in maintaining blood sugar.

Accordingly, kidney glycogen values were determined immediately, and 1½–2½ hr after evisceration and hepatectomy in normal and diabetic dogs. After evisceration, the blood sugar was allowed to fall spontaneously, since it has been under these conditions that the kidney has been shown to contribute glucose to blood. The techniques for pancreatectomy, evisceration, and hepatectomy, and for the chemical analysis of the specimens were described previously (*2, 3*).

TABLE 1

KIDNEY GLYCOGEN AND BLOOD GLUCOSE VALUES IN NORMAL AND DIABETIC EVISCERATED, HEPATECTOMIZED DOGS IMMEDIATELY AND AT VARIOUS TIME INTERVALS AFTER EVISCERATION

Normal dogs	Time after evisceration (min)	Kidney glycogen (g %)	Blood sugar (mg %)
No. 1	0	0.026	64
	70	0.030	16
No. 2	0	0.025	82
	120	0.028	23
No. 3	0	0.031	80
	105	0.043	20
Diabetic dogs			
No. 1	0	0.099	270
	120	0.109	144
No. 2	0	0.079	257
	150	0.108	135
No. 3	0	0.097	336
	125	0.103	170

As can be seen from Table 1, it is impossible to account for the glucose released by the kidney on the basis of the glycogen originally present in these organs. Thus the immediate postevisceration biopsies of kidneys reveal only about 0.05 g of glycogen/100 g of kidney tissue in normal dogs and about 0.1 g of glycogen/100 g of kidney tissue in diabetic dogs. If this glycogen were to serve as a source of blood sugar, the total amount present

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(approximately 50 mg) could in no way account for the amount of glucose contributed by renal tissue. As can be seen from the data, renal glycogen did not disappear but increased slightly in amount during the period glucose was being released into the blood stream. It is obvious, therefore, that the glucose contributed to blood by the kidney arises from noncarbohydrate precursors. The renal output of glucose is larger than can be attributed to temporary storage of glucose as glycogen with subsequent glycogenolysis and release of glucose to the blood stream.

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The Effect of Exercise upon the Lethality of Roentgen Rays for Rats

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The extent of biological damage resulting from exposure to roentgen rays can be altered by physical and chemical measures that alter the metabolic level of the test organism. The mortality rate of irradiated homeothermic animals can be increased by exposure to the physical stress of a hypothermic environment following irradiation (*6, 10*). Irradiated poikilothermic organisms, such as fertilized *Ascaris* eggs (*3*), frogs (*9*), chick embryos (*11*), and newborn rats (*4–6*), show evidence of greater resistance or repair when kept at low temperature. In several of these studies (*3–5, 11*), the lowered metabolic activity of the organism is indicated as the explanation for increased resistance to radiation. It is of interest to note that anoxic conditions favor survival of organisms and enhance tissue resistance to radiation (*1, 5, 8*).

Treatment of mice with thyroxine greatly increases the lethality of a given dose of ionizing radiation—an increased oxygen consumption was also demonstrated (*2*). Kirschner *et al.* (*7*) demonstrated that rats that died following potentially lethal doses of x-rays had higher postirradiation metabolic rates than those that survived, although both groups showed rises above normal.

Such studies suggest that conditions tending to increase the metabolic rate would also increase the lethal effects of roentgen rays. In order to investigate further the role of metabolic level in determining radiosensitivity, vigorous exercise was selected to provide a physiological means of increasing the metabolic rate as an alternative to stimulation by drugs or changes in the physical environment of the organism.

[1] The authors wish to acknowledge the technical assistance of J. L. Lee and T. A. Gonzalez, of the Naval Radiological Defense Laboratory, San Francisco Naval Shipyard.

TABLE 1

Total Mortality to Rats* for each Dose Level of X-Rays Delivered to the Whole Body

	Not irradiated, exercised	Irradiated, not exercised	Irradiated, exercised
600 r			
Initial No.	18	30	26
No. died	0	0	13
% Died	0	0	50
700 r			
Initial No.	26	38	26
No. died	0	13	24
% died	0	44	92
860 r			
Initial No.	21	30	33
No. died	0	25	33
% died	0	83	100

* Animals were observed for 90 days.

Male rats of Sprague-Dawley strain bred in this laboratory were used throughout this study. For each dose level studied, all animals were from litters whelped at approximately the same time and were of similar weight (±8%). The exercised animals performed a standardized exhaustive exercise test for rats, which was developed in this laboratory (*12*). In this test, they were allowed to swim individually in tanks of water until they were exhausted. The animals were exercised for ten trials prior to irradiation in order to adapt them to the conditions of testing. After irradiation they were exercised daily, five times per week throughout the study. At each radiation-dose level employed, nonirradiated animals were exercised concurrently with the irradiated animals, but no deleterious effects of the performance test per se were observed. The duration of exercise was found to be 15–30 min per trial with a weight load of 10 g attached to each animal.

Because the number of animals that could be exercised per day was limited, irradiation at each dose level was performed on different days and with slightly different radiation factors, as follows: Radiation factors for 600 r were: 250 kv x-rays, 15 ma; 1.3-mm copper filter; target distance, 27 in.; 25 r/min air dose. For 700 r and 860 r the factors were: 240 kv x-rays, 8 ma; 0.6-mm copper filter; target distance, 39 in.; 8.5 r/min air dose.

After irradiation with 600 r, 50% of the exercised rats died, and all nonexercised irradiated rats survived (Table 1 and Fig. 1). Exhaustive exercise of less than 30-min duration per day was sufficient to induce a 50% mortality at this dose of x-rays. At a dose (700 r) that was somewhat lethal (44%) to nonexercised animals, there was a doubling in the incidence of mortality (92%) among exercised animals. At a highly lethal dose (860 r), animals that were exercised showed symptoms of roughened coat, diarrhea, and crusted nares much sooner after irradiation than did their nonexercised controls. With

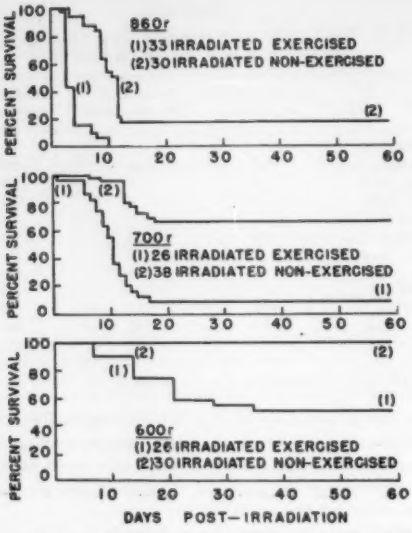

FIG. 1. Effect of exhaustive exercise upon postirradiation survival.

doses (700 r–860 r) that are, to a degree, lethal for nonexercised animals, the exercised animals had a much shorter postirradiation survival time, as well as a higher mortality rate.

As the animals were immersed in water during the period of exercise, it became necessary to know whether exposure to water without exercise would alter the mortality of irradiated rats. After a dose of 860 r, 30 irradiated rats were immersed to the neck in the swimming tanks 20 min daily for 3 weeks in wire containers that did not permit the animals to exercise, without altering the mortality rate from that of nonexposed irradiated rats. To determine whether exercise prior to irradiation would alter the mortality rate, a group of 20 rats, irradiated with 860 r, was exercised exhaustively for ten trials before irradiation and given no further treatment. The mortality rate was similar to that of nonexercised irradiated animals; hence it appears that the mortality rate is altered only if the animals are exercised after irradiation.

The increase in mortality with exercise following irradiation could not be correlated with alterations in total body weight. The time at which minimum body weight had been reached following radiation was similar for exercised and nonexercised animals. The weight loss was not sufficiently greater in exercised animals to provide evidence for a correlation between weight loss and increased mortality.

It is apparent that the lethality of roentgen rays at the doses studied (600 r, 700 r, and 860 r) is increased by the repeated performance of vigorous exercise following irradiation. It is suggested that the increased mortality observed with animals exercised after irradiation provides additional evidence of the relation between radiosensitivity and metabolic level.

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Adaptive Production of Amylase and Lipase by Three Species of Fungi[1]

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Recently, it was found that several bacteria and yeasts form certain enzymes adaptively (*4*, *6*, *9*). The present paper describes what appears to be adaptive production of amylase and lipase by three fungi.

Aspergillus flavus Link, *A. terreus* Thom, and a species of *Penicillium* tentatively identified as *P. notatum* Westling were grown at room temperature in 250-ml Erlenmeyer flasks containing 50 ml of medium. The basal medium for all the experiments was that of Czapek-Dox (*5*). Sucrose and soluble starch were compared as carbon sources of amylase production, and sucrose was compared with Mazola corn oil as a carbon source for lipase production.

Amylase. Twenty milliliters of a 1% agar solution containing 0.1% soluble starch and, as a preservative, 1 part formaldehyde in 1,500 parts of medium, was dispensed into each of several Petri dishes and allowed to harden. Filter paper disks 15 mm in diam were dipped into a solution or suspension of the material to be tested for amylase activity, the excess was drained off, and the disks were placed on the agar. Four disks were applied to each plate, three disks containing active material and one containing material heated in boiling water to serve as a control. The dishes were incubated at 30° C for 24 hr, and then were flooded with iodine-potassium iodide. The diameter of the clear zone around each disk was used as a measure of the amount of amylase present (*5*).

The amylase activities of the mycelium, and of the culture liquid in which the mycelium was growing, were tested. The mycelium was ground in several changes of

[1] Paper No. 2542, Scientific Series, Minnesota Agricultural Experiment Station.

acetone, and given a final wash in ether, as described by Tate (7), and the filter paper disks were dipped in a 1% suspension of this powder in distilled water. The culture liquid was tested without dilution.

Results of such tests with *A. flavus* and *A. terreus*, growing on 3% sucrose and on 2% starch, are given in Table 1.

TABLE 1

AMYLASE ACTIVITY* OF THE MYCELIUM AND CULTURE MEDIUM OF TWO FUNGI GROWN ON SUCROSE OR STARCH AS A CARBON SOURCE

Days after inoculation	*A. flavus*				*A. terreus*			
	mycelium		medium		mycelium		medium	
	sucrose	starch	sucrose	starch	sucrose	starch	sucrose	starch
5	0	+++	–†	–	0	++	–	–
8	0	++	+	+++	0	+	0	++
11	trace	+	–	–	trace	trace	–	–

* Rated 0 to +++ on the basis of size of zone as judged by eye but not measured.
† Not tested.

A commercial sample of Taka-diastase was diluted with distilled water to give solutions of 10%, 1%, 0.1%, 0.01%, and 0.001%, and these were similarly tested. The results are shown in Fig. 1, each point representing an average of 9 disks. The relationship between diameter of clear zone and enzyme concentration appears to be logarithmic.

Basal media containing 3% sucrose and 1% starch were mixed to give the following combinations:

Medium No.	Parts of: 3% Sucrose medium	1% Starch medium
1	100	0
2	80	20
3	60	40
4	40	60
5	20	80
6	0	100

These media were inoculated with *A. flavus*, and the medium and mycelium were periodically tested for amylase. The results for four of the media are presented in Fig. 2. Media 3 and 4 were essentially similar to 5. Amylase activity increased in both the medium and the mycelium as the amount of starch in the medium was increased. In cultures 34 days old, considerable amylase was still present in the medium, but only a trace was detected in the mycelium.

In another experiment, *A. flavus* was grown for 9 days on media containing different proportions of sucrose and starch. The culture liquid was then poured off, the mat was washed several times with sterile distilled water, and a 0.1% solution of starch, in distilled water containing formaldehyde in the proportion of 1:1,500, was introduced under the mats in the flasks (*2*). Aliquots of this solution were tested periodically with iodine-potassium iodide. The results, given in Table 2, confirm those obtained when paper disks were used.

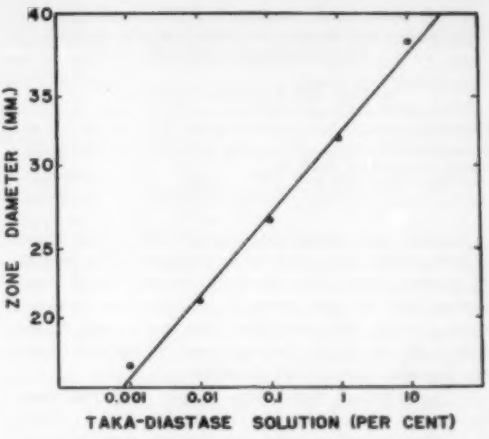

FIG. 1. Relationship between diameter of clear zone and concentration of Taka-diastase using paper-disk assay method.

Lipase. *A. flavus* and *Penicillium* sp. were inoculated into replicate 250-ml flasks containing 50 ml of the following media: (1) Three percent sucrose; (2) 1.5% Mazola corn oil; 0.1% sucrose. The 0.1% sucrose solution was added to the second medium to permit the mycelium

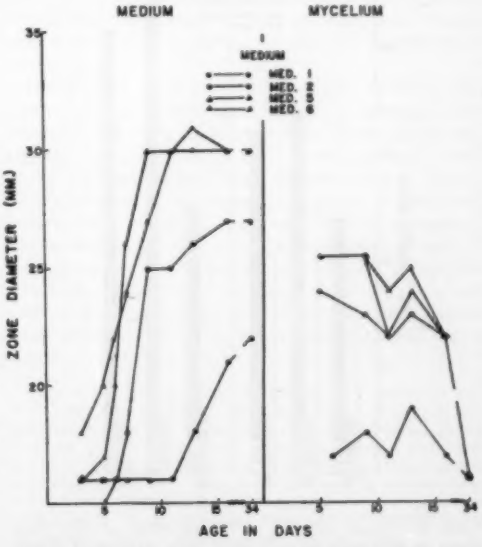

FIG. 2. Amylase activity of the culture medium and mycelium of *A. flavus* grown on media containing different proportions of sucrose and starch. See text for composition of media.

TABLE 2

HOURS FOR STARCH TO SHOW CHANGE FROM BLUE TO VIOLET WITH I-KI

Medium used for growth of fungus				
Percent sucrose	3	2	1	0
Percent starch	0	1	2	3
Hours for replaced solution to show color change	52	52	42	18

to become well established. Agar, 0.25%, was added to both media. The corn oil was emulsified by passing the medium several times through a hand-operated homogenizer, and the agar served to stabilize this emulsion. When a heavy, well-sporulating mat had formed on the surface of the liquid, the mycelium in replicate flasks was harvested and treated as follows:

1. The mycelial mats were ground with ten times their wet weight of 90% glycerol, as described by Fodor (*1*). The ground material was kept at room temperature for 24 hr and then centrifuged to remove most of the cell debris. The centrifugate was stored at 2° C until tested.

2. Acetone powders were prepared as described in the tests for amylase.

3. The mats were air-dried on blotting paper for 24 hr, and then were ground to a fine powder in a mortar. The powder, so prepared, and that prepared with acetone, were kept in a desiccator over $CaCl_2$.

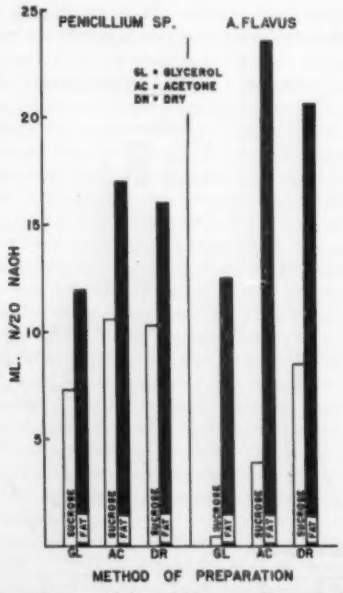

FIG. 3. Lipase activity of the mycelium of two fungi grown on sucrose or corn oil using three methods of preparing the mycelium for testing.

The substrate for testing lipase activity was similar to that described by Peters and Nelson (*3*), consisting of corn oil and 0.25% agar in a citrate-phosphate buffer at pH 6.5. This was dispensed in 10-ml amounts into 125-ml flasks. Three parts of the glycerol extracts were diluted with two parts of buffer before use, and 3 ml of this was added to each 10 ml of substrate. The dry powders were added at the rate of 0.05 g to 10 ml of substrate. Heated controls were included.

The substrate and enzymes were incubated for 48 hr at room temperature (about 22° C). At the end of this period, 50 ml of 1:1 ether-alcohol was added to each flask, and the fatty acids were titrated with $N/20$ alcoholic NaOH.

The results, representing the average difference between the heated control and the active preparation for three flasks of each treatment, are presented in Fig. 3.

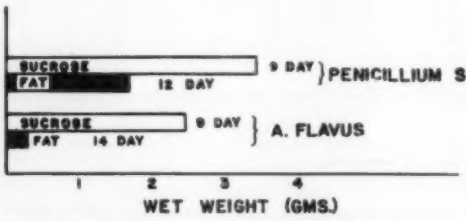

FIG. 4. Weight of mycelium of two species of fungi grown on sucrose or corn oil.

The powders are directly comparable with one another in amounts of fungus material added. It is estimated that the glycerol extracts represent only about ¼–⅓ the amount of fungus material present in the powders. The mycelium of both fungi, in all three methods of preparation, had a much higher lipase activity when grown on the medium containing corn oil than when grown on that not containing corn oil, although an appreciably greater weight of mycelium was produced on the medium not containing corn oil, as shown in Fig. 4.

It has been shown that amylase and lipase are produced adaptively by the fungi used in these tests, considerably more enzyme being produced when the organisms were grown in the presence of the specific enzyme substrated. This is in agreement with the work on adaptive hydrolytic enzymes in bacteria and yeasts. In the present studies enzyme activity was measured in the absence of growth, formaldehyde being used as an antiseptic. It is not known how long the specific activity would be retained had the substrate been changed and growth permitted to continue. The possibility of selection from a heterogeneous population of strains better adapted to a specific medium was not ruled out, and this possibility must be investigated.

The fungi used in these studies are commonly associated with the deterioration of stored grain, and are among several of the organisms being investigated in connection with such spoilage. The results suggest that enzymatic activities of such fungi may be influenced by the substrate on which the fungi are growing, and this may be of some significance in the field of grain deterioration, as well as in the field of commercial production of enzymes.

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Determination of Circulating Red Cell Volume by Radioactive Chromium[1]

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Previous investigators (*3–9*) have reported accurate methods of measuring the circulating red blood cell volume by the injection of red cells labeled with radioisotopes. Radioactive iron and radioactive phosphorus were available for these studies. The preparation of red cells tagged with radioactive iron necessitates the administration of radioiron for several weeks to volunteer donors who incorporate it into the hemoglobin of their red cells, which must then be transfused into the experimental subject (*3–5*). Radioactive phosphorus (*6–9*) may be used to label a small volume of the experimental subject's own red cells *in vitro*. However, the phosphate exchanges rapidly between the red cells and plasma, causing the radioactivity of the tagged cells to fall significantly after 1–3 hr (*6–9*).

The present study concerns a new biological tracer, radioactive chromium (Cr^{51}), with a half-life of 26.5 days. When this isotope is added to blood *in vitro* as $Na_2Cr^{51}O_4$, it is taken up avidly by the red cells, which retain their radioactivity without significant loss for periods of 1 day or more after injection into experimental animals. The uptake of Cr^{51} by human red cells is illustrated in Fig. 1, demonstrating the marked affinity of red cells for $Na_2Cr^{51}O_4$. Since the exchange of Cr^{51} between red cells and plasma is negligible for 24 hr, this isotope appears ideal for the tagging of red blood cells and the measurement of the circulating red cell volume.

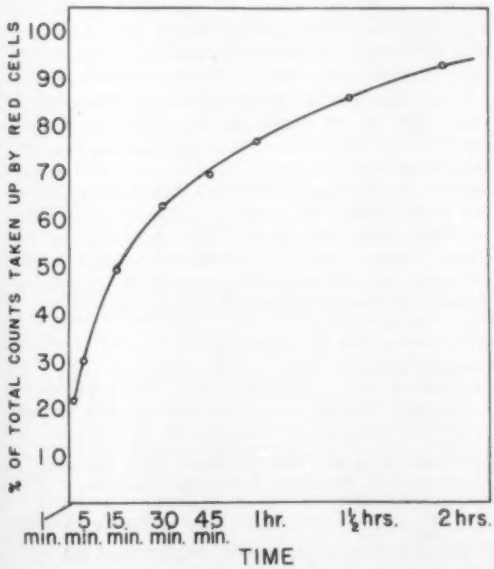

FIG. 1. Uptake of Cr^{51} as $Na_2Cr^{51}O_4$ by human red blood cells; 86 μg chromium added to 2 ml packed red cells suspended in saline at 24° C.

Approximately 50 ml of whole blood was withdrawn from a normal mongrel dog and heparinized. Cr^{51} (7–26 μc),[3] as $Na_2Cr^{51}O_4$, was added to whole blood or a red cell suspension in saline. After an hour, the red cells were washed with either physiological saline or plasma and then resuspended in plasma from the same dog. The plasma radioactivity was less than 1% of the radioactivity of the red cells.

A 4-ml aliquot was removed for counting, and the remaining measured volume of blood containing tagged red cells was injected intravenously into the dog. After allowing time for mixing in the circulation, 4-ml samples were withdrawn for counting at intervals varying from 10 min to 24 hr.

The injected blood and all subsequent blood samples were centrifuged in 4-ml hematocrit tubes, and the plasma was separated. One milliliter of each plasma sample was pipetted into a weighed planchet, dried overnight at 60° C (to constant weight) and counted with a Geiger-Müller counter. The plasma, regardless of the time interval, had no significant counts above background. The packed red cells were dried overnight at 60° C, then ground to a fine powder and counted in weighed planchets, preferably in duplicate. Since Cr^{51} is a soft x-ray emitter, self-absorption corrections were applied to all samples. All counts were corrected for radioactive decay.

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[2] The authors wish to thank Arthur K. Solomon and Charles B. Robinson for their assistance in the processing and counting of the radioactive isotopes.

[3] The radioactivity dosage required for this method is well within the tolerance limits for human use. From the disintegration scheme of H. Bradt *et al.* (*1*), the administration of 42 μc of Cr^{51} per kg body wt approximates a total dose of 1 rep (*2*), on the conservative assumptions that there is no excretion and that radiation is localized in the red cells. The amount of chromium injected per kg body wt varied from 1 μg to 20 μg, depending upon the specific activity of the sample. This is well below the toxicity level.

In order to obtain the high specific activity required for this research, samples of chromium, enriched in Cr^{50}, were bombarded in the nuclear reactor. We wish to express our thanks to the Isotopes Division of the Atomic Energy Commission for making this material available.

The counts per ml of packed red cells were obtained by computing the counts in 0.35 g of dried ground red cells, since this value was found to be equivalent to 1 ml of packed red cells.

The circulating red cell volume of the animal is given by the formula:

Red cell volume in ml =

$$\frac{\text{total counts injected}}{\text{counts per ml packed red cells in sample}}. \quad (1)$$

The numerator of this fraction is obtained from the counts per ml of packed red cells, the volume of whole blood injected, and the hematocrit.

TABLE 1

DETERMINATION OF RED CELL VOLUME WITH CHROMIUM AS A RADIOACTIVE TRACER

Dog No.	Sex	Wt (in kg)	Red cell volume in ml*				Mean (in ml)	Standard deviation (in %)
			10–30 min	20–60 min	30 min–3 hr	18–24 hr		
6	♀	11.6	332	330	345	344	338	2.3
10	♀	17.5	477	450	495	503	481	4.9
12	♂	14.0	442	442	436	—	440	0.8
13	♀	13.2	446	436	441	458	445	2.1
15	♂	0.0	353	368	372	380	368	3.1

* Determinations based on consecutive blood samples.

Table 1 lists the measurements of red cell volume obtained from consecutive blood samples after allowing time for mixing of the tagged cells. The values are in close agreement with standard deviations of 0.8%–4.9% for periods of 18–24 hr. The activity of the red cell samples declined gradually thereafter. The plasma continued to show no significant activity.

The accuracy of the method was demonstrated by a second determination after transfusion or hemorrhage of a known volume of red cells. The dogs were transfused with approximately 400 ml of their own untreated blood withdrawn earlier. Additional blood samples were obtained following the transfusion, and the activity of the circulating red cells, now diluted by the addition of the untagged blood, was determined. The circulating red cell volume was then calculated according to equation (1).

After hemorrhage, however, the radioactivity of the circulating red cells remained unaltered, since hemorrhage represents the removal of an aliquot of blood containing an unaltered proportion of tagged and untagged red blood cells. Consequently, a second injection of tagged red cells was necessary to verify the red cell volume after hemorrhage. As before, this is given by the formula:

Red cell volume in ml =

$$\frac{\text{total counts in circulation}}{\text{counts per ml packed red cells in sample}}. \quad (2)$$

The numerator here represents the sum of the counts of the two injections of tagged cells minus the counts removed by the hemorrhage.

TABLE 2

RED CELL VOLUME DETERMINATION BY Cr^{51} BEFORE AND AFTER HEMORRHAGE OR TRANSFUSION

Dog No.	Cr^{51} Determinations		Calculated change in red cell volume (in ml)	Measured hemorrhage or transfusion (volume of whole blood × hematocrit) (in ml)	Difference (in %)
	Initial red cell volume (in ml)	Final red cell volume after hemorrhage or transfusion (in ml)			
7	382	228	−154	−148	4.1
8	300	480	+180	+171	5.3
9	360	538	+178	+179	0.6
11	678	466	−212	−215	1.4
14	386	527	+141	+141	0

Table 2 shows five such experiments in which the changes in red cell volume, as determined by the Cr^{51} method within a 24-hr period, were in good agreement with the actual volumetric measurement of the hemorrhage or transfusion with a difference of 5.3% or less.

Determination of the circulating red cell volume by Cr^{51} shares with the P^{32} method the advantage that a small sample of the subject's own blood may be tagged rapidly *in vitro* and then reinjected. In contrast to P^{32} tagging, however, the Cr^{51}-tagged cells retain their activity without loss to the plasma for periods of 1 day or longer after injection, which fact should prove advantageous for clinical studies. After 3 days, the red cell activity decreases by approximately 15%; a similar fall is observed within 3 hr when P^{32}-tagged red cells are used (*6–9*).

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Comments and Communications

Failure of Vitamin B_{12} to Promote Growth of Premature Infants

With the development of knowledge of animal protein factors and of vitamin B_{12} as growth-promoting factors for bacteria and some animals, it seemed that possibly these agents might be of value in similarly promoting the growth of premature infants.

A study was undertaken in this clinic several months ago to determine whether or not this could be demonstrated. To date, that portion of the study having to do with the use of vitamin B_{12} intramuscularly has been completed.

Forty-eight infants with birth weight between 1,245 g and 2,226 g have been considered. Twenty-five received vitamin B_{12}[1] intramuscularly, and the others served as controls. The average birth weight in each of the groups was similar, 1,950 g and 1,933 g, respectively, for those receiving the vitamin and the controls. All infants received one of three types of formula, with comparative distribution between control and experimental groups.

TABLE 1

	Controls	Vitamin B_{12}
Average birth weight	1,933 g	1,950 g
Average discharge weight	2,630 "	2,577 "
Average total gain	698 "	618 "
Average daily gain	29.4 "	24.5 "
Average time to regain birth weight	7.5 days	8 days
Average time to reach 2.5 kg:		
Birth weight 1,500–2,000 g	28.6 "	29.3 "
Birth weight 2,000–2,500 g	14.6 "	14.8 "

The general condition of the infants in each group was similar. The vitamin B_{12} was given intramuscularly in a dosage of 10 μg. The frequency of administration varied from daily to every three days. The average total dose was 70 μg, with a range of 30–220. The time of introduction of the substance varied from the second to the seventh day of life, the average being the fourth day.

The results of the study in this particular group of premature infants was disappointing. There was no appreciable difference between those receiving vitamin B_{12} and those serving as controls in respect to average total gain before discharge, average daily gain, average time to regain birth weight, and average time to reach a weight of 2.5 kg. Indeed, on all counts there was a slight margin of superiority in the control infants. No difference was noted in the individual infants in the experimental group so far as dosage was concerned, those receiving the maximum total dose gaining no better than those receiving the minimal amount.

Although the group studied is not large, one may perhaps justly conclude that vitamin B_{12} is not effective in promoting weight gain of what might be termed normal premature infants. One may speculate that larger doses intramuscularly or doses similar to those used, but given orally, might be more effective. These possibilities are being explored, but we doubt that the results will differ materially from those detailed above. Wetzel (Wetzel, N. C., Fargo, W. C., and Smith, I. H. *Science*, 1949, **110**, 651) has recently reported suggestive evidence that the vitamin is effective in promoting weight gain in certain older children with slow progress in growth, children who perhaps have a deficiency of the factor. It is probably reasonable to believe that no premature infant will benefit from its administration unless he, too, has the specific deficiency. At present there is no evidence that such deficiency exists in premature infants.

DANIEL F. DOWNING
The Hahnemann Medical College and Hospital of Philadelphia

[1] Cobione, kindly supplied by Merck and Company.

Effect of Tricaine Methanesulfonate on the Determination of Sulfonamides

Tricaine methanesulfonate was used with a considerable success for the anesthesia of lower vertebrates (McGovern, B. H., and Rugh, R. *Proc. Soc. Biol. Med.*, 1944, **57**, 127; Hamburger, V. *A manual of experimental embryology*. Chicago: Univ. Chicago Press, 1942. Part II, p. 40; Schotte, O. E., and Harland, M. *J. Morphol.*, 1943, **73**, 329; and Gordon, M. N. Y. Zool. Soc. Private communication). Therefore, in one of the series of tests on the concentration of sulfamerazine in the tissues of fingerling brook trout (*Salvelinus fontinalis*), tricaine methanesulfonate M.S. 222 (Sandoz) was used as an anesthetic. The results in this series became inconsistent with the previous tests, and the tissue level of sulfamerazine was higher than could be expected. Therefore, tests were carried out in order to determine the effect, if any, of the anesthetic on the sulfonamide test.

Sulfamerazine was determined with a photometric modification of the method of Bratton and Marshall (Bratton, A. C., and Marshall, E. K. *J. biol. Chem.*, 1939, **128**, 537). The trout selected for the determinations were expected to contain in their tissues only a trace of sulfamerazine or none. In the trout anesthetized with tricaine methanesulfonate, the test indicated that the tissue level of free sulfamerazine was from 1 to 2 mg % and of total sulfamerazine from 2 to 4 mg %. Trout from the same batch, but not anesthetized, gave negative results, indicating that they were free from this sulfonamide.

Anesthesia was carried out by dipping trout for less than 1 min in a solution of tricaine methanesulfonate 1 : 5,000 in spring water.

This observation shows that tricaine methanesulfonate, or other anesthetics with similar molecular structure, should not be used whenever they may interfere with the colorimetric tests for sulfonamides.

S. B. Friddle and S. F. Snieszko
U. S. Fish and Wildlife Service, Kearneysville, West Virginia

Book Reviews

Einführung in die Kernphysik. 4th rev. ed. Wolfgang Riezler. Berlin and Buxtehude, Germany: Hermann Hübener, 1950. 309 pp.

The chief difficulty in introducing nuclear physics to the beginner or to the worker in other branches of the natural sciences is to find a scientifically correct presentation requiring a minimum of background in physics. This is accentuated if, as the author wishes, the text should also appeal to a wider public. Riezler attacks the problem by giving an essentially nonmathematical, somewhat narrative treatment, using the historical approach for balanced measure. Formulas are used sparingly. Even where they are given, the nonphysicist is generally able to understand the result from the text alone.

The first half of the book contains a survey of the physical aspects in a deductive order: atomic physics, general properties of nuclei, radioactivity, nuclear reactions, elementary particles, and systematics and structure of nuclei. The second, in parts more specialized, is devoted to nuclear techniques and to the applications of nuclear physics. Tables of isotopes and nuclear reactions, as of summer 1949, a glossary, and registers fill the last fifty pages. A two-tone isotopic chart, giving abundances and half-lives only, is attached.

The book is, throughout, well written, clear, and usefully illustrated. The small number of printing errors and factual mistakes will be detected and corrected by most readers. A few instances of repetitions of topics are excusable. One major omission may be noted. The liquid drop model is not mentioned, although the packing fraction curve and the mass parabolas for isobars are given and discussed. The chapter on electronics of nuclear detection instruments is rather inadequate, at least for the reader in the United States, since several outmoded designs are presented. The usefulness of the extensive tables is seriously limited by the lack of references. A list of only about 40 books and review articles is given in the bibliography, without connection to the tables or to the text. Finally, in the chart of isotopes, one may object to the distinction made between positron emitters and nuclei decaying by positron emission *and* electron capture.

These are all minor objections, however, to a text which gives an excellent survey of nuclear physics for beginners and nonphysicists. It is even quite fruitful and pleasant reading—in good German—for the physicist. It may be added that the print, though fine, is very clear, on good paper, and that the book is well bound, in gratifying contrast to some volumes received from Germany during or shortly after the war.

E. Bleuler
Purdue University

Kleine Raketenkunde. Hans K. Kaiser. Stuttgart, Germany: Mundus-Verlag, 1949. 150 pp. DM 9.

This is not the only new book on rockets and rocket research that has been published in Western Germany since the end of hostilities. There have been three so far, but *Kleine Raketenkunde* is certainly the most impressive of them. In external appearance it compares well with better books of German manufacture of prewar times and is almost lavishly illustrated. Many of the photographs collected by its author are rather rare, a number never having been published in a book before. Hans Kaiser was the founder of a prewar rocket society, devoted to space travel ideas, which had the courage to continue the work of the original German Rocket Society in spite of frowns from Nazi headquarters. Naturally, that society did not last very long, and its founder was then put to work in the research institute at Peenemünde, where the V-2, the *Wasserfall*, the *Taifun*, and a number of other rockets were created.

Since the book is meant for the general reader, it logically begins with an elementary explanation of the third law of motion and its application to rockets. This is followed by a chapter on rocket research, most of it in Germany, during the period from 1920 to 1930. Then there is a chapter on modern solid fuel rockets, followed by one on German work on liquid fuel rockets during the second world war. This chapter contains a good deal of information that will be news to virtually every reader, in Germany as well as in all other countries.

The author then continues his narrative of contemporary rocket history with an account of postwar work—this largely in the United States—as far as the sources at his disposal permit. The last chapter is devoted to the future. A list of the more important publications in book form and a list of technical terms with explanations form appendices. Some readers who did not know anything about rockets until V-2 appeared on the scene may be surprised by the heavy stress throughout the book on space travel. But these ideas are what started rocket research in Germany.

Willy Ley
Montvale, New Jersey

News and Notes

Upper Atmosphere Symposium and Conference

Warren N. Arnquist

Office of Naval Research, Pasadena, California

Under the auspices of the California Institute of Technology, the Naval Ordnance Test Station, and the Office of Naval Research, Department of the Navy, a symposium on upper atmosphere physics was held at the California Institute of Technology, Pasadena, on May 15. This was followed by a two-day conference on this subject at the Naval Ordnance Test Station (Inyokern) China Lake, California.

These meetings reflect, in general, the interest in this field in the Southern California area and, in particular, the influence of three outstanding European scientists who are in residence there. Sidney Chapman, of Oxford University, who has contributed extensively in many problems of the upper atmosphere, began a one-year stay at the California Institute of Technology in April, under an arrangement made possible by W. H. Pickering and his Signal Corps project. Marcel Nicolet, chief of the Department of Radiation, Royal Meteorological Institute of Belgium, and David R. Bates, of University College, London, have been actively associated with the ONR-sponsored light-of-the-night-sky research program under C. T. Elvey and F. E. Roach at the Naval Ordnance Test Station. Dr. Nicolet is noted for his work on the ionosphere, and Mr. Bates has made outstanding contributions concerning the photochemical reactions in the upper atmosphere.

The symposium consisted of nine papers which covered the field from the structure of the atmosphere to cosmic rays at very high altitudes. Approximately 80 people attended. Dr. Chapman spoke on "Theories of the Aurora," Dr. Nicolet on "Formation of the Ionized Layers," and Mr. Bates on "Basic Reactions in the Night Sky Emission." J. A. Van Allen, of the Applied Physics Laboratory, presented experimental results which have been obtained with rockets in his paper on "Cosmic Rays at Very High Altitudes," and A. B. Meinel, of Yerkes Observatory, gave an account of his recent experimental work in a paper entitled "OH Emission Bands in the Spectrum of the Night Sky," and also read a paper on the same subject by Gerhard Herzberg, of the Canadian National Research Council. R. Penndorf, of the Air Force Cambridge Research Laboratory, J. Kaplan and S. W. Kash, of the University of California at Los Angeles, and W. W. Kellogg, of UCLA and the Rand Corporation, also presented papers.

Perhaps the most significant contributions that were reported for the first time in this meeting had to do with the interpretation of Dr. Meinel's experimental identification of the rotational bands of OH in the "airglow," the night sky radiation other than the aurora, the zodiacal light, and the twilight. This interpretation was exhaustively treated by Bates and Nicolet and independently by Herzberg. It appears that the basic reaction responsible for the OH lines is the one between atomic hydrogen and ozone at an altitude of about 70 km. This height has been confirmed experimentally by Dr. Roach, using a specially designed recording photometer, which sweeps the sky in a series of azimuths. The atomic hydrogen comes from the dissociation of water vapor by sunlight.

On May 16–17 about 20 of these scientists held informal round table conferences on upper atmosphere physics at the Naval Ordnance Test Station. Three sessions were held under the chairmanship of C. T. Elvey, Sidney Chapman, and J. L. Greenstein, of the California Institute of Technology. The discussions included the altitudes of nocturnal and twilight emissions and of the aurora, the identification of spectral lines in the airglow and the aurora, the temperature, density, and other aspects of the composition of the atmosphere, the entry of corpuscles into the atmosphere and their emission from the sun, the theories of the aurora, the solar ultraviolet absorption in the atmosphere, and the specific reaction mechanisms in the upper atmosphere. Besides the scientists already mentioned, those who attended this conference included M. A. Tuve and L. V. Berkner of the Carnegie Institution of Washington, Oliver Wulf, B. Gutenberg, and M. H. Nichols of CIT, Marcus O'Day, of the Cambridge Research Laboratories, G. L. Weissler, of USC, Fred Rogers, Jr., and E. V. Ashburn of NOTS, and W. N. Arnquist.

Many recommendations for further work were made during the course of these meetings. It was generally agreed that rockets should be used more extensively as vehicles for carrying instrumentation to high altitudes and that the study of the night sky emissions should continue, because of the directness of such approaches in contrast to such indirect methods as the propagation of sound or the scattering of light by particles. Also, the cost of useful rocket flights is becoming more reasonable with the development of smaller rockets. Rockets can also be used to carry tracer substances to appropriate altitudes for release in order to study the effects produced; for example, Dr. Roach has deduced that the sodium radiation of the night sky comes from an altitude between 250 and 300 km, whereas Mr. Bates predicts theoretically that this radiation should come from about 70 km. In discussing this situation, no way could be found to harmonize theory and experiment. Consequently, an experiment was recommended to release sodium vapor from a rocket at these altitudes in order to observe the enhancement, if any, of this radiation with instrumentation on the ground. In addition, it was con-

sidered that appropriate photometers should be mounted in rockets so as to study directly the natural emissions themselves as a function of altitude. In discussing the aurora, the most important recommendation was to make more height determinations by the two-station triangulation method for correlation with spectroscopic data.

All the members of this conference had very favorable reactions concerning its value. The meeting attempted to review not only the ideas that have become reasonably well established, but also the problems that are puzzling workers in this field and the schemes that are evolving in regard to new problems to be tackled or new ways of undertaking old problems. Many people felt that other meetings of this character should be encouraged, especially in those fields that have received marked stimulation as a result of the instrumentation developments of the past few years. It was considered that the size of the meeting had much to do with its character. With the number involved, it was possible to maintain an informal atmosphere; on the other hand, the representation in the field of upper atmosphere physics was sufficiently broad for all problems that came up. The dinner given by the commanding officer of NOTS served to develop personal relationships and to provide an opportunity for numerous personal discussions, many of which were continued in the ensuing sessions.

About People

Gerald Berman, of Toronto, Ontario, has been appointed instructor of mathematics at Illinois Institute of Technology, and **William E. Thompson**, associate research mechanical engineer, Cornell Aeronautical Laboratory, Buffalo, New York, has been appointed instructor in mechanical engineering.

Bart J. Bok, associate director of Harvard College Observatory, who is spending the year at the observatory's southern station in South Africa, delivered the Rubbi Lecture at Stellenbosch University. Later he addressed the Stellenbosch Student Society on "The Milky Way," and a joint meeting at Capetown on "The Age of the Universe." The Rubbi Lecture, given in Dutch, was on the dynamics of star clusters.

Norris E. Bradbury, director of the Los Alamos Scientific Laboratory since October, 1945, has been appointed professor of physics at the University of California, Berkeley. Dr. Bradbury will continue as director of the laboratory at Los Alamos.

Robert Broh-Kahn, formerly assistant director of the May Institute for Medical Research, Cincinnati, has been appointed assistant medical director of Bristol Laboratories, Syracuse, New York. For the past two years Dr. Broh-Kahn was also assistant professor of experimental medicine at the College of Medicine, University of Cincinnati.

R. Keith Cannan, professor of chemistry at New York University, will become chairman of the Committee on Growth, of the National Research Council, to succeed **Milton C. Winternitz**, new head of NRC's Medical Sciences Division. **Hayden C. Nicholson** is resigning as executive secretary of the committee, to go to the University of Arkansas as dean of the School of Medicine.

D. Frank Holtman, who has spent the past year as chief of the Microbiology Branch, Biological Department, Chemical Corps, Camp Detrick, Maryland, has returned to his position as professor and head of the Department of Bacteriology, University of Tennessee.

Marion J. Partridge has been appointed assistant supervisor in the Physical Therapy Section, Department of Physical Medicine and Rehabilitation, at the University of Illinois Research and Educational Hospitals. Miss Partridge was a member of the staff of the Veterans Administration Hospital, Castle Point, New York.

S. R. M. Reynolds, of the Department of Embryology, Carnegie Institution of Washington, will be at the Nuffield Institute for Medical Research, Oxford, after September 5. He will work with staff members in research on the character of blood flow in umbilical arteries and veins, until July, 1951.

E. W. R. Steacie has been appointed vice president of the Canadian National Research Council. Dr. Steacie will be responsible for the coordination of all scientific activities of the organization, and for integration of research throughout its laboratories. He will continue his present research in chemical kinetics as director of the Division of Chemistry.

Cecil Legriel Wittson has been appointed professor of neurology and psychiatry at the University of Nebraska College of Medicine and director of the Nebraska Psychiatric Unit, a teaching unit of the College of Medicine and the School of Nursing. Dr. Wittson was formerly associated with the Central Islip State Hospital, New York.

Visitors

A. Tybjaerg Hansen, of the Rigs Hospital, Copenhagen, an international authority on the intravascular pressures in vascular disease, visited Walter Reed General Hospital and Army Medical School, Washington, D. C., August 1–10.

C. C. Hu, professor of pomology, Taiwan University, Taihoku, Formosa, is a visitor at the University of California's College of Agriculture Agricultural Experiment Station, Riverside. Dr. Hu, a specialist in citrus and subtropical fruits, will spend a year in this country studying citrus fruits.

Grants and Awards

The Wisconsin Alumni Research Foundation, which administers patents obtained on discoveries made by University of Wisconsin scientists, has made a grant of $633,008 to the university to be used for research grants-in-aid, symposia and conferences, scientific apparatus, research

assistants, the Charles Sumner Slichter professorship, the University of Wisconsin Press, and for amortization of special building loans.

The 1950 Joseph Goldberger Award in Clinical Nutrition, made annually by the American Medical Association, has been presented to Fuller Albright, associate professor of medicine at Harvard Medical School. Dr. Albright received the award for his work in metabolism.

Creighton University School of Medicine, Omaha, Nebraska, has received a grant of $6,000 from the **Office of Naval Research,** for an investigation of the effect of methyl blue on respiration of mammalian erythrocytes, using the Warburg technique, and $500 from the **Council on Pharmacy and Chemistry of the American Medical Association,** for study of the effect of anticonvulsants on metabolism of the brain, also using the Warburg technique. Both investigations will be carried on by H. C. Struck, assistant professor of physiology and pharmacology.

The Elliott Cresson Medal of the Franklin Institute, Philadelphia, has been awarded to Basil F. J. Schonland, president of the South African Scientific and Industrial Research Organization, Johannesburg, for research in atmospheric electricity and experimental investigations of lightning.

Fellowships

The National Research Council's Committee on Growth, acting for the American Cancer Society, is accepting applications for grants and fellowships. Applications for new grants will be accepted until *October 1*; final decisions will be made early next year and will become effective July 1, 1951. Investigators now receiving grants will be notified individually regarding applications for extension.

Fellowships in Cancer Research of the American Cancer Society, and Damon Runyon Clinical Research Fellowships, financed by a grant from the Damon Runyon Memorial Fund to the American Cancer Society, are available. Fellowship applications may be submitted at any time. Those received before November 1 will be acted upon by the committee in December; those received between November 1 and March 1 will be acted upon in April. Fellowships will ordinarily begin July 1, although this date may be changed at the request of the applicant. All communications should be addressed to the Executive Secretary, Committee on Growth, NRC, 2101 Constitution Avenue, Washington 25, D. C.

During the past year the American Cancer Society, on recommendation of the Committee on Growth, has awarded grants and fellowships approximating $2,000,000. A program of similar magnitude is planned for the coming year.

Colleges and Universities

Northwestern University will sponsor a series of weekly lectures on inorganic chemistry during the fall quarter, to be given at Lincoln Hall. The lectures will constitute a noncredit course, and will be open to all, for the usual course fee. Speakers on the program and the dates of their lectures are: September 26—J. W. Kennedy, Washington University, St. Louis; October 3—W. A. Weyl, Pennsylvania State College; October 10—Michael Fleischer, U. S. Geological Survey; October 17—G. H. Cady, University of Washington; October 24—W. M. Latimer, University of California, Berkeley; October 31—A. B. Burg, University of Southern California; November 7—Linus Pauling, California Institute of Technology; November 14—J. J. Grebe, Dow Chemical Company; November 21—Jacob Kleinberg, University of Kansas; and November 28—G. W. Watt, University of Texas.

The Johns Hopkins University has received $10,000 from Charles F. Kettering, research consultant of General Motors Corporation, to establish a postdoctoral research fellowship in the McCollum-Pratt Institute for a three-year period. The fellowship will be used to support the work of a young scientist for research and training in the field of trace elements.

Indiana University recently established a Graduate Institute for Applied Mathematics, which at present will deal primarily with problems in gas dynamics, hydrodynamics, and turbulence. Students entering the institute are required to have at least one year of graduate work in mathematics, and the M.A. degree is desirable. Research assistantships are open to students who wish to study for the Ph.D. degree. C. A. Truesdell, of the Naval Research Laboratory, Washington, D. C., will be visiting professor for 1950–51. Further information may be obtained from T. Y. Thomas, Head, Graduate Institute for Applied Mathematics, Indiana University, Bloomington.

The University of Tennessee College of Medicine has made the following appointments to its staff: Sigfrid Zitzlsperger, instructor in anatomy; Fern W. Smith, assistant professor of anatomy; Frederick Meyers, instructor in pharmacology; James E. Alexander, assistant in medicine; McCarthy DeMere, Edward French, and William T. Tyson, Jr., assistants in surgery. John Ifft, Boston University College of Medicine, is visiting associate professor of anatomy this summer.

Meetings and Elections

The Medical Women's International Association will hold its sixth congress in Philadelphia, September 10–16, at Women's Medical College of Pennsylvania, which is celebrating its centennial. Ninety-five women medical delegates from 15 foreign countries are expected to attend.

A national noise abatement symposium, sponsored jointly by the National Noise Abatement Council and the Armour Research Foundation of Illinois Institute of Technology, will be held October 20, in Chicago. Haldon A. Leedy, director of the foundation, is chairman of the program committee.

The Royal Society of Canada held its annual meeting at the Royal Military College, Kingston, Ontario,

June 5–7. Officers for 1950–51 are: president, J. J. O'Neill, McGill University; honorary secretary, Séraphin Marion, National Archives, Ontario; honorary executive secretary, W. H. Cook, Canadian National Research Council; president, Section III, H. G. Thode, Ontario; president, Section IV, P. S. Warren, University of Alberta; and president, Section V, L. S. Simard, University of Montreal.

The Connecticut Agricultural Experiment Station, oldest in the country, will celebrate its 75th anniversary in New Haven, Connecticut, September 28–29. Some 3,000 scientists, agriculturists, and officials of government, universities, experiment stations, and industry have been invited to attend.

Open house will be held for delegates and the public on Thursday, the 28th, and will include special demonstrations of work carried on at the station. Detlev W. Bronk, president of Johns Hopkins University, and Arnold Nicholson, managing editor of *Country Gentleman*, will address the visitors.

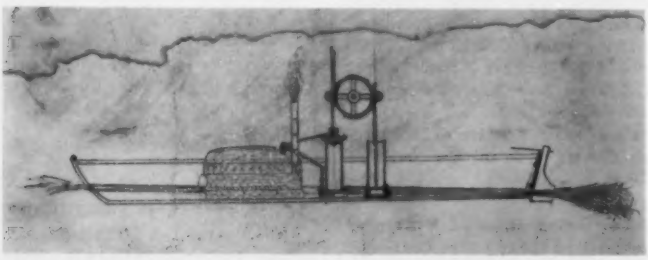
A symposium on "The Research Institute in Modern Society" will be held on Friday, with Edmund W. Sinnott, director of the Sheffield Scientific School and dean of the Graduate School at Yale University, serving as moderator. Various aspects of the topic will be discussed by George O. Curme, Jr., vice president in charge of chemical research, Union Carbide and Carbon Corporation, New York City; Selman W. Waksman, head of the Department of Microbiology, New Jersey Agricultural Experiment Station; Alexander Wetmore, secretary, Smithsonian Institution; and Elvin C. Stakman, chief of the Division of Plant Pathology and Botany, University of Minnesota. Their respective subjects will be industrial research, governmental institutes, endowed institutes, and universities. Speakers at the Friday evening dinner will include Chester Bowles, governor of the state and chairman of the station's Board of Control, who will serve as toastmaster; C. R. Orton, director emeritus, West Virginia Agricultural Experiment Station, representing the Association of Land-Grant Colleges and Universities; Dr. Bronk, representing the National Academy of Sciences; Walley Taylor, assistant agricultural attaché, British Embassy, representing the Rothhamsted Experimental Station of England; George A. Baitsell, professor of biology, Yale University, representing the AAAS.

A national collection of industrial bacteria was begun at the Chemical Research Laboratory, Department of Scientific and Industrial Research, London, early this year. It contains some 350 types, including the nonpathogenic cultures formerly held by the National Collection of Type Cultures at Colindale, England. The laboratory will maintain any organism that has ceased to be of interest, but which may be wanted later. It will also maintain any organism that should be duplicated. Most of the nonpathogenic bacteria supplied up to now by Colindale can be obtained from the Chemical Research Laboratory, and the laboratory will try to procure any type of nonpathogenic culture that it does not hold. It is hoped that the collection will become comprehensive. Gaps are being filled by obtaining cultures from laboratories in England and other countries. Particular emphasis will be given to assay organisms.

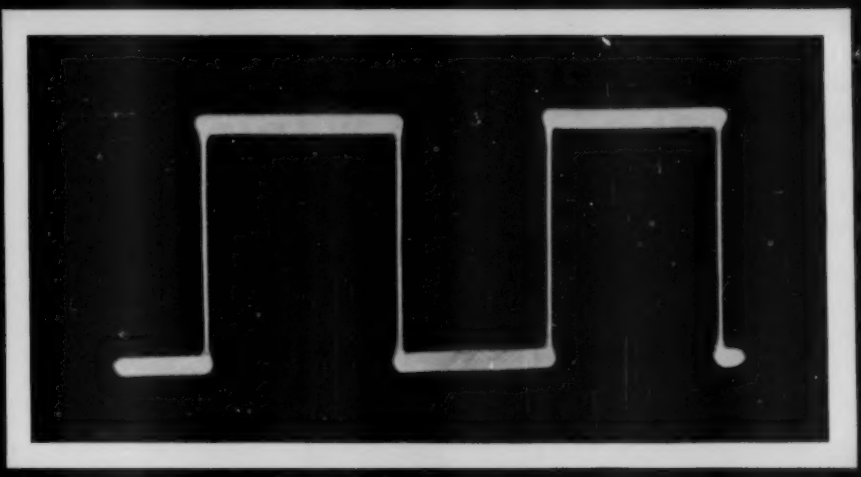
Cultures held in the collection will be included in the *United Kingdom List of Species*, to be published in 1951. Inquiries and requests for cultures should be addressed to the Director, Chemical Research Laboratory, Teddington, Middlesex, England.

The Alaska field station of the Public Health Service (*Science*, April 28, p. 480) has been officially named the **Arctic Health Research Center,** with headquarters at Anchorage. Investigations to date have revealed echinococcosis infection in both wild and domestic animals, trichinosis among arctic marine animals and carnivora, and a high incidence of fish tapeworm among natives in fish-eating areas. Research will be continued on these problems, and control measures are being worked out, particularly in regard to many previously unstudied biting insects of the region. Jack C. Haldeman is officer in charge.

The U. S. Department of Agriculture has sent Franklin S. Harris, president emeritus of Utah State Agricultural College, and Hoyt Turner, specialist in agricultural extension and education, to Tehran to assist the government of Iran in a program of agricultural improvement. Dr. Harris was agricultural advisor to Iran in 1939–40. Conservation and better use of limited water supply, control of pests and diseases of crops and livestock, increased fertility of the land, better utilization, transportation, and marketing of farm produce, introduction of improved crops and livestock, and use of labor-saving methods on farms will be studied.

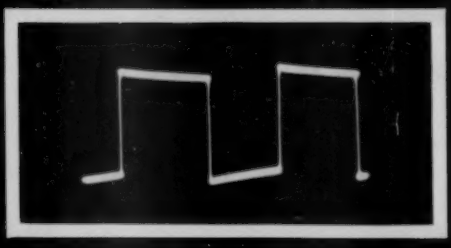


This eighteenth-century drawing by John Fitch of a jet-propelled boat is one of more than 150 items in the "Milestones in American Achievement" exhibition that opened at the Library of Congress on July 18. The contributions of 34 scientists, and of other prominent Americans, ranging from Henry Adams to John Peter Zenger, and extending from the discovery of America to the end of World War I, are represented by some memento taken from the collections of the Library. As part of Washington's Sesquicentennial Celebration, the exhibition will be on view until December 31, 1950, and a catalogue of its contents will be issued this fall.

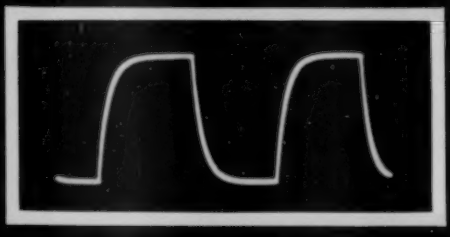


A perfect square wave, photographed by engineers of Allen B. DuMont Laboratories, Inc., at the output of a high-frequency amplifier. This is the result of repeated adjustment and readjustment of a compensated attenuator and peaking coils.

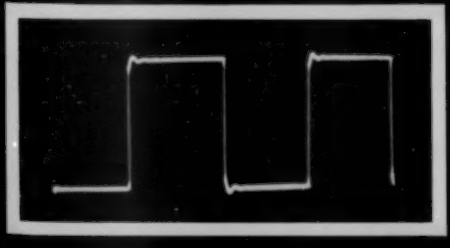
Improper adjustment results in poor low-frequency response. Note tilt in top and bottom flats. Percentage of tilt is a measure of low-frequency response and low-frequency phase shift.



Effect of "under peaking" of high-frequency compensating inductances. Note that rise time of square wave has been distorted so that the leading edge is rounded instead of sharp.



"Over peaking" with extremely fast rise. This produces "ringing" in the leading edge of the square wave.



PHOTOGRAPHY

helps adjust an amplifier

Far subtler differences than shown here can have large effects on performance.

How can you remember the all-important details of wave form? How can you show improvements achieved in the course of design changes and adjustments? How can you prove that a circuit long since gone from your bench behaved in a certain way?

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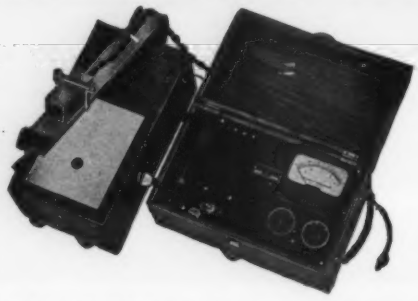

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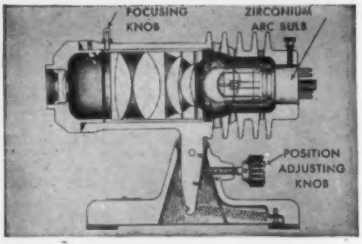

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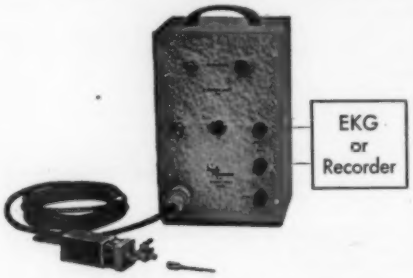

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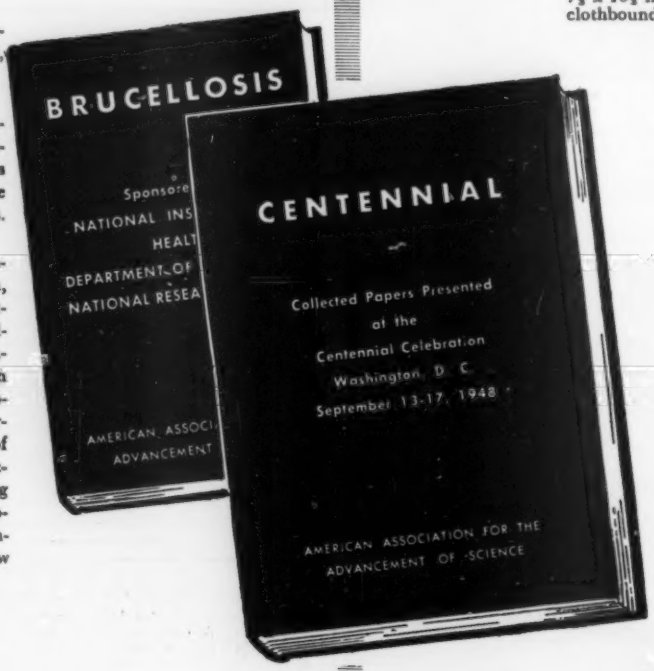

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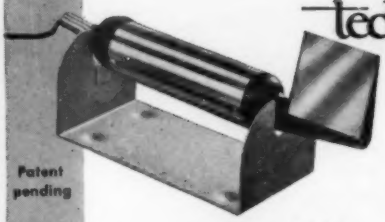

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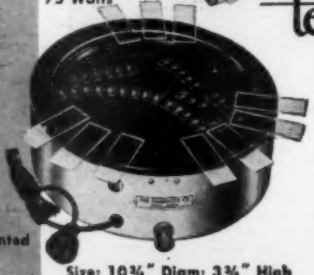

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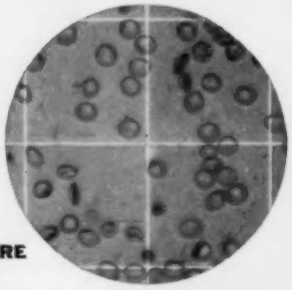

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